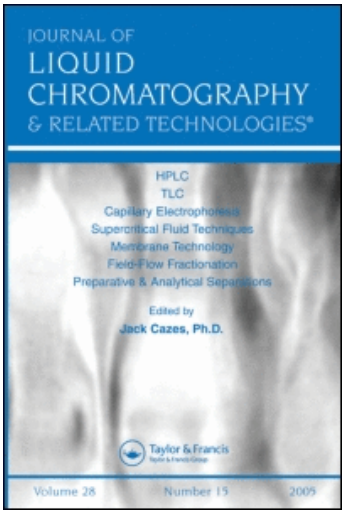

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HPLC Determination of Dacarbazine, Doxorubicin, and Ondansetron Mixture in 5% Dextrose Injection on Underivatized Silica with an Aqueous-Organic Mobile Phase

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HPLC DETERMINATION OF DACARBAZINE, DOXORUBICIN, AND ONDANSETRON MIXTURE IN 5% DEXTROSE INJECTION ON UNDERIVATIZED SILICA WITH AN AQUEOUS-ORGANIC MOBILE PHASE

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ABSTRACT:

A high performance liquid chromatography procedure has been developed for the assay of a dacarbazine, doxorubicin, and ondansetron mixture in 5% dextrose injection. The separation and quantitation are achieved on an 22-cm underivatized silica column at ambient temperature using a mobile phase of 60:40 v/v 6.25 mM phosphate buffer, pH 3.0 - acetonitrile at a flow rate of 1.0 ml/min with detection of all three analytes at 216 nm. The separation is achieved within 10 min with sensitivity in the ng/ml range for each analyte. It was shown that the predominant mechanism of retention for the analytes on silica was cation exchange. The method showed linearity for dacarbazine, doxorubicin, and ondansetron in the 0.79-7.90, 0.08-1.60, and 0.06-6.00 μg/ml ranges, respectively. Accuracy and precision were in the 0-7% and 0.4-6% ranges, respectively, for all three compounds. The limits of detection for dacarbazine, doxorubicin, and ondansetron were 12.5, 10.0 and 8.0 ng/ml, respectively, based on a signal to noise ratio of 3 and a 50 μl injection.

INTRODUCTION

A mixture of dacarbazine, 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 5% dextrose injection required the development of an HPLC method. A search of the literature indicated that a HPLC method was not available to assay for all three compounds concurrently in a single injection.

Dacarbazine has been previously analyzed by spectrophotometry (1,2), electrochemistry (3), and HPLC (4,5). The spectrophotometric procedure is used as the official USP XXII assay for both the drug substance and injection. The electro-chemistry of dacarbazine has also been studied using differential pulse polarography, adsorptive stripping voltammetry, and oxidative amperometry at a glassy carbon electrode. All three electrochemical methods were compared and applied to the HPLC determination of dacarbazine in serum. The assays were designed for serum analysis and used an octadecylsilane column with aqueous-organic mobile phases to detect the drug in the low μg/ml range.

Assay methods for doxorubicin have included spectrophotometry (6), electrochemistry (7,8), microbiological agar diffusion (6), HPLC (9-12), and TLC (13). 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 (12).

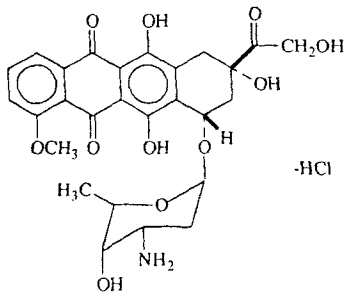
Ondansetron has been assayed by high performance thin-layer chromatography (HPTLC) and HPLC methods (14-16). The HPTLC method was developed especially for plasma samples, but the sample throughput was low and the equipment is not generally available in most laboratories. The HPLC assays used either a 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 dacarbazine, doxorubicin, and ondansetron in a mixture using a single injection. The compounds are separated on underivatized silica using a buffered aqueous acetonitrile eluent. The separation is achieved within 10 min at ambient temperature with sensitivity in the ng/mL range.

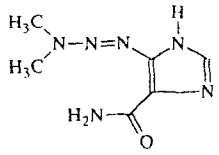
EXPERIMENTAL

Reagents and Chemicals:

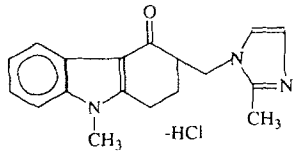
The structure formulae of the compounds studied are shown in Figure 1. Dacarbazine and doxorubicin were purchased from the United States Pharmacopeial Convention, Inc. (Rockville, MD 20852). Ondansetron (Batch C662/116/1) was a gift from Glaxo, Inc. (Research



DOXORUBICIN HCl



DACARBAZINE



ONDANSETRON HCl

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 (Contenental Water Systems, Roswell, GA 30076). Monobasic sodium phosphate and concentrated phosphoric acid 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 50 μl loop, a Kratos Model 757 variable wavelength UV-VIS detector (Ramsey, NJ 07446) and a Hewlett-Packard Model 3394A integrator (Avondale, PA 19311). Separation was accomplished on a 22 cm silica column (4.6mm i.d., 5 μm particle size, Brownlee Labs, Santa Clara, CA 95050). The mobile phase consisted of 60:40 v/v 6.25mM aqueous monobasic sodium phosphate, pH 3.0 (adjusted with concd. phosphoric acid)- acetonitrile. The mobile phase was filtered through a 0.45 μm Nylon-66 filter (MSI, Westborough, MA) and degassed by sonication prior to use. The flow rate was set at 1 ml/min. The UV detector was set at 216 nm.

Preparation of Standard Solutions:

A combined standard solution containing dacarbazine, doxorubicin, and ondansetron was prepared by accurately weighing 7.89 mg, 0.78 mg, and 0.62 mg of each powder calculated as the free base, transferring to a 100-ml volumetric flask, manually shaking for 10 min and 70:30 acetonitrile-water added to volume. Dilutions (1:25, 1:50 and1:100) were made in the aqueous acetonitrile mix from the standard solution to obtain solutions containing 3.16, 1.58 and 0.79 μg/ml of darcarbazine, 0.31, 0.16 and 0.08 μg/ml of doxorubicin, and 0.25, 0.12 *and 0.06*

μg/ml of ondansetron. Three point calibration curves were constructed for each analyte. Additional dilutions (1:33.3 and 1:66.7) of the combined standard solution were prepared in aqueous acetonitrile 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 dacarbazine, doxorubicin and ondansetron mixture in 5% dextrose 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 reverse phase systems using octadecylsilane columns with various mobile phases containing acetonitrile-or methanol-aqueous phosphate buffers (pH 3.0). In almost every system studied, the dacarbazine peak eluted at or near the solvent front followed in order by ondansetron and doxorubicin. The latter two compounds showed retention on most brands of octadecylsilane column investigated with the

doxorubicin peak having retention times greater than 30 min on most columns. Band broadening of both ondansetron and doxorubicin peaks was observed. The dacarbazine peak was usually sharp and symmetrical although some tailing was observed on one stationary phase based on an ODS spherical silica particle. In one experiment, an ion-pairing agent (pentanesulfonic acid, sodium salt) was added to the mobile phase to especially aid in the retention of dacarbazine. Unfortunately, the dacarbazine peak did not show increased retention and the ondansetron and doxorubicin peaks were not eluted within 30 min. The addition of more water to the mobile phase only moved the dacarbazine peak slightly away from the solvent front, but at the expense of extremely long retention times for ondansetron and doxorubicin (>25-30 min). Therefore, it appeared that ODS systems would not be appropriate for separating the three structurally different analytes in this study.

Our attention then turned to the use of an underivatized silica column with a buffered aqueous-organic mobile phase for the separation and quantitation of these analytes. This laboratory has previously reported HPLC methods to analyze basic, acidic and neutral compounds in pharmaceutical dosage forms and biological samples using underivatized silica (17-19). The separation mechanism for basic drugs with buffered aqueous mobiles phases has been ascribed to the interaction of silanols with an amine group to produce a cation exchange mechanism. Since there were no reports in the scientific literature

describing the separation of our basic drug mixture on silica, we investigated several mobile phases differing in pH, ionic strength and concentration, and type of organic modifier.

The pH of the mobile phase was varied from 2.5 to 7.5. Initial trials showed that pH did not affect the retention of any of the analytes. It has been our experience with silica columns that they show shorter equilibration times, less prominent solvent fronts and are much more stable when operated at low pH. Therefore, the mobile phase pH used in this study was set at 3.0.

The ionic strength of the phosphate buffer component of the mobile phase was varied between 0.004 to 0.008u. It was found that the ionic strength of the mobile phase was the predominant parameter affecting retention of the analytes. Increasing the ionic strength significantly decreased retention times. A 6.25 mM buffer strength was selected for the assay based on the best resolution of the three analytes within a reasonable chromatographic run time The low concentration of buffer also aided in preventing excessive pump seal wear.

The concentration of acetonitrile in the mobile phase had little or no effect on retention times. As the acetonitrile composition was increased from 30 to 50% of the mobile phase, the retention of each analyte essentially remained the same. Substituting methanol for acetonitrile caused a significant effect on retention. As methanol composition in the mobile phase increased from 30 to 50%, dacarbazine

and ondansetron showed increased retention and doxorubicin showed decreased retention. The effect of methanol on the retention of ondansetron was particularly significant since the retention was approximately 4 times longer than with acetonitrile in the mobile phase.

Therefore, it appeared from our studies that the predominant mechanism of retention of these basic analytes on undervatized silica was cation exchange. The addition of methanol to the mobile phase probably replaced water held in the third and even second layer adsorbed to silica and caused differences in retention due to the increased lipophilicity of the stationary phase as compared to equal amounts of acetonitrile in the mobile phase. This effect of methanol versus acetonitrile has been previously reported by our laboratories in the separation of non-steroidal anti-inflammatory agents and anabolic steroids on underivatized silica [18,19].

Thus, a mobile phase consisting of 60:40 v/v 6.25 mM phosphate buffer pH 3.0 - acetonitrile was selected for the assay. A typical chromatogram of the three analytes is shown in Figure 2 .

In the acetonitrile-phosphate buffer mobile phase, the absorption maxina for dacarbazine, doxorubicin, and ondansetron were 238, 254, and 216 nm, respectively. It was decided to use 216 nm as the detection wavelength since ondansetron was present at the lowest concentration in the drug mixture and the other two drugs also showed significant absorption at that wavelength.

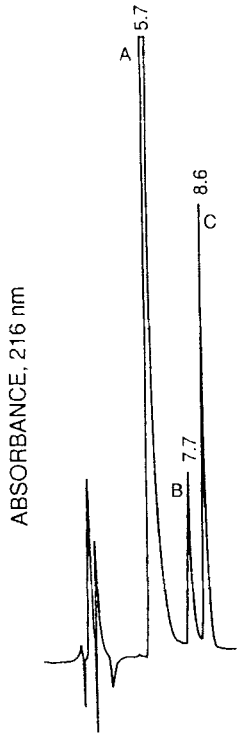


Figure 2 - Typical HPLC chromatogram of dacarbazine (A), doxorubicin (B), and ondansetron (C) on underivatized silica with acetonitrile - aqueous phosphate buffer pH 3.0 mobile phase. See Experimental Section for assay conditions.

The HPLC method showed concentration versus absorbance linearity for dacarbazine, doxorubicin, and ondansetron in the 0.79-7.90, 0.08-1.60 and 0.06-6.00 μg/mL ranges, respectively at 216 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

Table 1

Analytical Figures of Merit For Dacarbazine, Doxorubicin, and Ondansetron on Underivatized Silica

Analyte	r^{2a}	System Suitability[b]	LOD[c] ng/ml	k^1	Theoretical Plates[d]	Tailing Factor[e]	Rs
Dacarbazine	0.9997	0.71	12.5	1.8	1046	1.6	
							5.0
Doxorubicin	0.9999	1.64	10.0	2.6	3730	1.1	
							2.4
Ondansetron	0.9999	0.89	8.0	3.1	14680	1.1	

[a]Range examined from 0.79-7.9 μg/mL dacarbazine (n = 10) 0.08-1.6 μg/mL doxorubicin (n = 12), and 0.06-6.0 μg/mL ondansetron (n = 14)Mobile phase consisted of 60:40 6.25 mM phosphate buffer, pH 3.0-acetonitrile at 1.0 ml/min with detection at 216 nm.
[b]RSD % of 6 replicate injections at 3.2 μg/mL dacarbazine, 0.31μg/mL doxorubicin, and 0.25 μg/mLondansetron at 216 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		Percent Error	RSD (%)
	Added (μg/mL)	Found[a] (μg/mL)		
Dacarbazine	2.10	2.09 ± 0.015	4.76	0.72
	1.05	1.03 ± 0.004	1.90	0.39
Doxorubicin	0.208	0.203 ± 0.007	2.40	3.45
	0.104	0.111 ± 0.005	6.73	4.45
Ondansetron	0.165	0.164 ± 0.001	0.61	0.61
	0.083	0.083 ± 0.005	0.00	6.02

[a] Based on n = 3.

products of any of the three analytes would interfere with the quantitation of each drug at 216nm. These experiments were performed on solutions of the three drugs in 5% dextrose injection after they had been degraded for 6 hr at 80°C in both 0.1N acid and 0.1N 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.

Intra-day variability of the assay for dacarbazine, doxorubicin, and ondansetron expressed as % RSD was 0.8, 2.5 and 1.6% (n = 4), respectively. Inter-day variability of the assay for these drugs was 0.8, 2.2 and 1.2% (n = 12 over 4 days), respectively.

In summary, an underivatized silica column with an aqueous pH 3.0 buffer-acetonitrile mobile phase. has been shown to be amenable for the separation and quantitation of a dacarbazine-doxorubicin-ondansetron mixture in 5% dextrose injection. The HPLC method has advantages of using simple and inexpensive mobile phases and a comparatively inexpensive and very stable silica column. This study suggests that this HPLC method can be used to investigate the chemical stability of all three drugs in dextrose injection.

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