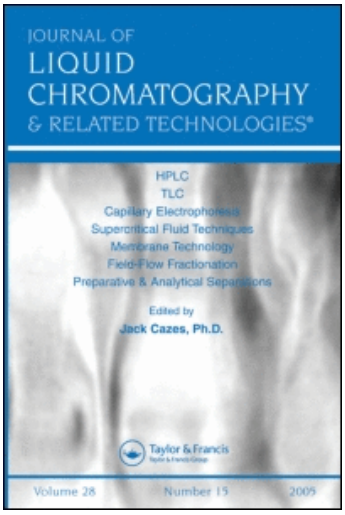

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HPLC DETERMINATION OF IDARUBICIN-ETOPOSIDE AND IDARUBICIN-ONDANSETRON MIXTURES IN 0.9% SODIUM CHLORIDE INJECTION USP

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ABSTRACT

High performance liquid chromatographic methods have been developed for the assays of idarubicin-etoposide and idarubicin-ondansetron mixtures in 0.9% sodium chloride injection USP. The separation and quantitation of the idarubicin-etoposide mixture was achieved on an octylsilane column at ambient temperature using a mobile phase of 73:27 v/v 0.02 M aqueous monobasic sodium phosphate - acetonitrile at a flow rate of 1.0 mL/min with UV detection at 254 nm. The separation was achieved within 13 min. The method showed linearity for idarubicin hydrochloride and etoposide in the 2 - 32 and 10 - 160 μg/mL ranges, respectively. Accuracy and precision were in the 0.1 - 2.9% and 0.1 - 2.1% ranges, respectively, for both analytes.

The separation and quantitation of the idarubicin-ondansetron mixture was accomplished on an octadecylsilane column at ambient temperature using a mobile phase of 57:43 v/v 0.02 M aqueous monobasic potassium phosphate - acetonitrile at a flow rate of 1.8 mL/min with UV detection at 254 nm. The separation was achieved within 15 min. The method showed linearity for idarubicin hydrochloride and ondansetron in the 2.4-38 and 2.3-37 μg/mL ranges, respectively, for both analytes.

INTRODUCTION

Mixtures of idarubicin-etoposide (Mixture A) and idarubicin-ondansetron (Mixture B) are highly effective in the treatment of certain types of cancer. 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 stability and compatibility 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 either Mixture A or Mixture B.

Idarubicin is a widely used anticancer drug. The assay methods recently reported for idarubicin are all HPLC based procedures.[1-6] The methods involve the separation of idarubicin on phenyl, cyanopropyl, ethylsilane, and octadecylsilane columns. The official USP 23 assays for idarubicin drug substance and injection dosage form utilize reverse-phase chromatography on a trimethylsilane column.[7]

Etoposide is also an important antineoplastic agent. It has been analyzed primarily by HPLC methods. The assays are based on reverse-phase separations on octadecylsilane, octylsilane, and phenyl columns with electrochemical or UV detection.[8-11]

Ondansetron is an antiemetic drug used for the prevention of nausea and vomiting including that associated with cancer chemotherapy. It has been analyzed by a variety of methods, such as HPLC,[12-14] spectrophotometry,[15-16] and radioimmunoassay[17] methods. The spectrophotometric determinations are based on either the formation of an ion-pair complex or a solid-phase reactor. The HPLC methods use silica, phenyl, octylsilane, ethylsilane, or cyanopropyl columns with aqueous phosphate and acetonitrile or methanol as mobile phase.

In this paper, isocratic HPLC assays are presented that will simultaneously analyze for idarubicin-etoposide (Mixture A) and idarubicin-ondansetron (Mixture B) mixtures in 0.9% sodium chloride injection USP using a single injection.

Each mixture is separated on an alkylsilane column using an aqueous phosphate - acetonitrile eluent. The separations are achieved within 13 min at ambient temperature.

EXPERIMENTAL

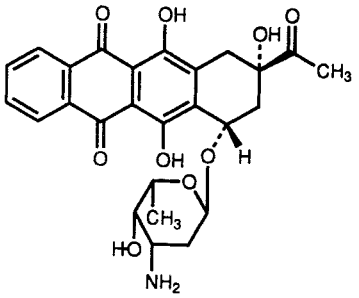
Reagents and Chemicals

The structure formulae of the compounds studied are shown in Figure 1. Idarubicin hydrochloride was purchased from the United States Pharmacopeial Convention, Inc. (Rockville, MD 20852). Etoposide powder was supplied by Bristol-Myers Squibb (Princeton, NJ 08543). Ondansetron was a gift from Glaxo-Wellcome (Research 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 and potassium phosphates were J.T. Baker analyzed reagent (Phillipsburg, NJ 08865).

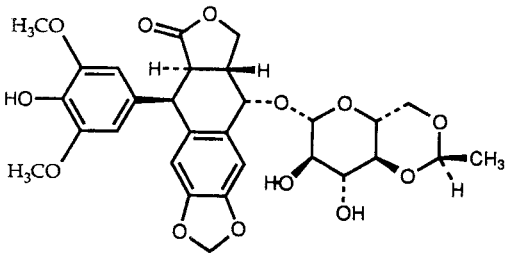
Instrumentation

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 ultraviolet-visible wavelength detector (Model Lambda Max 481, Waters Associates, Milford, MA 01757), and an HP Model 3394A Integrator (Hewlett-Packard Company, Avondale, PA 19311).

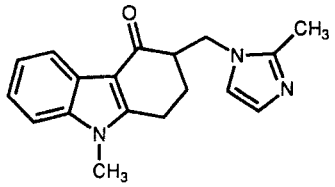
The separation of Mixture A was accomplished on an octylsilane column (Symmetry C8, 150 mm × 3.9 mm i.d., 5 μm particle size, Waters Chromatography, Milford, MA 01757) at ambient temperature (23 ± 1°C). The mobile phase consisted of 73:27 v/v 0.02 M aqueous monobasic sodium phosphate - acetonitrile.



Idarubicin



Etoposide



Ondansetron

Figure 1. Chemical structures of analytes studied.

The separation of Mixture B was achieved on an octadecylsilane column (Spherisorb ODS-2, 250 × 4.6 mm i.d., 5 μm particle size, Alltech Associates, Deerfield, IL 60015) at ambient temperature. The mobile phase consisted of 57:43 v/v 0.02 M aqueous monobasic potassium phosphate - acetonitrile.

The mobile phases were filtered through a 0.45 μm nylon-66 filter (MSI, Westborough, MA 01581) and degassed by sonication prior to use. Flow rates were 1.0 and 1.8 mL/min for Mixture A and Mixture B, respectively. Injection volumes were 20 μL and the detector was set at 254 nm for both mixtures.

Preparation of Standard Solutions

A combined standard solution for Mixture A was prepared by adding accurately weighed quantities of 0.84 mg of idarubicin hydrochloride and 4.1 mg of etoposide into a 25-mL volumetric flask, and adding 0.9% sodium chloride injection USP to volume. This combined standard solution along with appropriate dilutions of the solution using the sodium chloride injection gave solutions containing 2.1, 8.4, and 33.6 μg/mL of idarubicin hydrochloride and 10.2, 41.0, and 164 μg/mL of etoposide. The same process was used to prepare a combined standard solution and dilutions for Mixture B to obtain solutions containing 2.4, 9.4, and 38 μg/mL of idarubicin hydrochloride and 2.3, 9.2. and 37 μg/mL for ondansetron. Three point calibration curves were constructed for each analyte in their respective mixtures and additional dilutions in 0.9% sodium chloride injection USP were prepared to serve as spiked samples to determine accuracy and precision of the method for each of the analytes. Quantitation was based on linear regression analysis of peak area versus analyte concentration in μg/mL.

RESULTS AND DISCUSSION

The goal of this study was to develop HPLC assays using isocratic conditions for the analysis of an idarubicin-etoposide or idarubicin-ondansetron mixture in 0.9% sodium chloride injection USP. A stability study of each mixture would require an assay which would detect and quantitate each analyte with reasonable accuracy and precision.

There were no reports in the scientific literature describing concurrent separation and quantitation of either idarubicin-etoposide or idarubicin-ondansetron in a mixture. Initial studies to develop an HPLC method for each mixture using isocratic conditions involved the use of phenyl, methylsilane, octylsilane, and octadecylsilane columns with various mobile phases containing methanol and/or acetonitrile - aqueous phosphate at 1 mL/min.

The best resolution of the analytes in Mixture A was obtained on an octylsilane column using 73:27 v/v aqueous 0.02 M sodium phosphate - acetonitrile. The octylsilane column also allowed the separation of the two analytes from benzyl alcohol, a preservative found in etoposide injection. Mixture B was best resolved on an octadecylsilane column using 57:43 v/v aqueous 0.02 M potassium phosphate - acetonitrile. The octadecylsilane column also separated the two analytes from methylparaben and propylparaben, preservatives found in ondansetron injection. The other columns tested did not provide enough peak resolution of these preservatives.

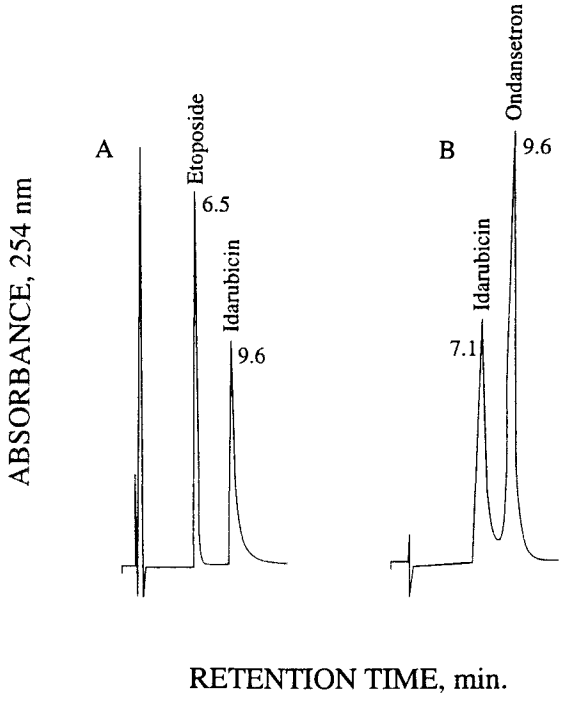


Figure 2. Typical HPLC chromatograms of the idarubicin-etoposide mixture (A) and the idarubicin-ondansetron mixture (B). See Experimental Section for HPLC assay conditions.

Typical chromatograms showing the separation of each analyte in the two mixtures are shown in Figure 2. The detection wavelength of 254 nm was selected for both assays since it provided good accuracy and precision data for each two component mixture.

It was found that 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 idarubicin much more significantly than those of the other analytes. Also, as the concentration of acetonitrile in the mobile phase increased, the retention of each analyte decreased. Therefore, aqueous 0.02 M phosphate was selected and different acetonitrile concentrations were used in the mobile phase to obtain the best separation of idarubicin from the other analyte in their respective mixture in the shortest run time.

Table 1

Analytical Figures of Merit for Idarubicin-Etoposide and Idabrubicin-Ontansetron Mixtures

Mixture	Conc. Range (μg/mL)	r^{2a}	System Suitability[b]	k'	N[c]	Tailing Factor[d]	Rs
A) Idarubicin hydrochloride	2.1-33.6	0.9998	1.80	7.6	1741	1.7	
							4.6
Etoposide	10.2-164	0.9999	0.69	4.8	3856	1.2	
B) Idarubicin hydrochloride	2.3-37	0.9998	1.00	6.6	1385	1.3	
							2.1
Ondansetron	2.4-38	0.9998	1.60	4.7	547	1.0	

[a] n=6. [b] Mean RSD% of 6 replicate injections of 21.0 μg/mL idarubicin HCl and 20.4 μg/mL etoposide for Mixture A and 19.0 μg/mL idarubicin HCl and 18.0 μg/mL ondansetron for Mixture B. [c] Calculated as $N = 16(t/w)^2$. [d] Calculated at 5% peak height.

The HPLC methods showed concentration versus absorbance linearity for the analytes at 254 nm. Table 1 gives the analytical figures of merit for each analyte 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 shown in the tables indicated that accuracy and precision for both procedures were in the 0.1 - 2.9% and 0.1 - 2.1% ranges, respectively, for the analytes in the two mixtures studied.

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	Idarubicin hydrochloride	3.99 15.78	3.94 ± 0.03 16.05± 0.47	0.1 1.7	0.8 2.9
	Etoposide	20.57 82.27	20.46 ± 0.08 82.67 ± 0.41	0.5 0.5	0.4 0.5
B	Idarubicin hydrochloride	4.70 18.82	4.80 ± 0.09 18.75 ± 0.20	2.1 0.4	1.9 1.1
	Ondansetron	4.53 18.14	4.37 ± 0.09 18.00 ± 0.42	3.6 2.3	2.0 0.8

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

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	Idarubicin hydrochloride	3.99 15.78	3.86 ± 0.11 15.89 ± 0.11	2.1 0.7	2.9 0.7
	Etoposide	20.57 82.27	20.50 ± 0.12 82.92 ± 0.33	0.3 0.8	0.6 0.4
B	Idarubicin hydrochloride	4.70 18.82	4.80 + 0.09 18.75 ± 0.20	1.9 1.1	2.1 0.4
	Ondansetron	4.53 18.14	4.37 ± 0.09 18.00 ± 0.42	2.0 2.3	3.6 0.8

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

In summary, an octylsilane column with an aqueous phosphate-acetonitrile mobile phase for the idarubicin-etoposide assay and an octadecylsilane column with an aqueous phosphate - acetonitrile mobile phase for the idarubicin-ondansetron assay have been shown to be amenable for the separation and quantitation of these analytes in 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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