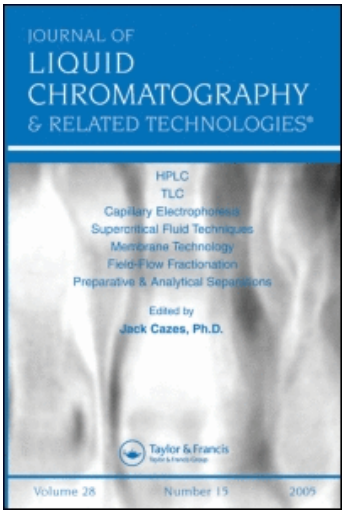

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HPLC Determination of an Ondan-Setron and Diphenhydramine Mixture in 0.9% Sodium Chloride Injection

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HPLC DETERMINATION OF AN ONDANSETRON AND DIPHENHYDRAMINE 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 an ondansetron hydrochloride and diphenhydramine hydrochloride mixture in 0.9% sodium chloride injection. The separation and quantitation were achieved on a 5-μm Spherisorb ODS-1 column at ambient temperature using a mobile phase of 60:40 v/v 0.1 M phosphate buffer pH 4.5-acetonitrile at flow rate of 1.2 mL/min. with detection of both analytes at 210 nm. The separation was achieved within 22 min. with sensitivity in the ng/mL range for each analyte. The method showed linearity for ondansetron and diphenhydramine in the 0.40 - 6.40 and 5.0 - 80.0 μg/mL ranges, respectively. Intra- and inter-day RSD values were 1.8% and 2.8 - 3.8% for ondansetron, and 1.4 - 1.7% and 2.0 - 2.7% for diphenhydramine, respectively. Accuracy of intra and inter-day were in the 1.0 - 1.6% and 1.2% for ondansetron and 0.7 - 2.0% and 0.3 - 3.8% for diphenhydramine, respectively. The limits of detection for ondansetron and diphenhydramine were 70 and 105 ng/mL, respectively, based on a signal to noise ratio of 3 and a 20 μL injection.

INTRODUCTION

A mixture of ondansetron hydrochloride and diphenhydramine hydrochloride can be administered as a perioperative injection in a hospital operating room. 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 both compounds concurrently with a single injection.

Ondansetron has been assayed by high performance thin layer chromatography (HPTLC) and HPLC methods.[1-3] The HPTLC method was developed especially for plasma samples, but the sample throughout 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.

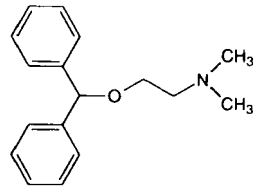
Assay methods for diphenhydramine hydrochloride have included spectrophotometry,[4-9] HPLC[10-13] and GC.[14,15] The HPLC methods are the most common of the procedures reported and have involved the separation of the drug on an octadecylsilane column. The official USP 23 assay for diphenhydramine hydrochloride injection utilizes reverse phase chromatography on a nitrile column.[16]

In this paper, an isocratic HPLC assay is presented that will simultaneously analyze ondansetron and diphenhydramine hydrochlorides in 0.9% sodium chloride injection using a single injection. The compounds are separated on an octadecylsilane column using a buffered aqueous - acetonitrile eluent. The separation is achieved within 22 min. at ambient temperature with sensitivity in the ng/mL range.

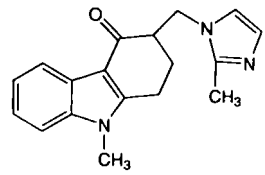
EXPERIMENTAL

Regents and Chemicals

The structure formulae of the compounds studied are shown in Figure 1. Diphenhydramine hydrochloride was purchased from Parke, Davis (Morris Plains, NJ 07950). Ondansetron hydrochloride (Batch C662/116/1) was a gift from Glaxo, Inc. (Research Triangle Park, NC 27709). Methyl paraben and propyl paraben were purchased from Sigma Chemical Co. (St. Louis, MO



DIPHENHYDRAMINE



ONDANSETRON

Figure 1. Chemical structures of compounds studied.

63178). 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 potassium phosphate was Baker analyzed reagent.

Instrumentation

The chromatographic separation was performed on an HPLC system consisting of a Beckman Model 110B Solvent Delivery Module (San Ramon, CA 94583), an ABI Model 759A UV-VIS Variable Wavelength Detector (Foster City, CA 94404) and an HP Model 3392A Integrator (Hewlett-Packard Company, Avondale, PA 19311). Separation was accomplished on a 5-μm Spherisorb ODS-1 column (250 x 4.6 mm i.d. Keystone, Bellefonte, PA

16823) equipped with a direct-connect ODS guard column at ambient temperature (23 ± 1°C). The mobile phase consisted of 60:40 v/v 0.1 M monobasic potassium phosphate pH 4.5 - 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 1.2 mL/min. and the detector was set at 210 nm.

Preparation of Standard Solutions

A combined standard solution containing ondansetron hydrochloride and diphenhydramine hydrochloride was prepared by accurately weighing 1.0 mg of ondansetron hydrochloride and 12.5 mg of diphenhydramine hydrochloride, transferring to a 10-mL volumetric flask, manually shaking for 10 min. and 0.9% sodium chloride injection containing 1.2 mg/mL of methyl paraben and 0.15 mg/mL of propyl paraben added to volume. Dilutions (8:125, 4:125 and 1:250) of the standard solution were made in mobile phase to obtain mixtures containing 6.4, 3.2 and 0.40 μg/mL of ondansetron, and 80.0, 40.0 and 5.0 μg/mL of diphenhydramine hydrochloride. Three point calibration curves were constructed for each analyte. Additional dilutions (1:19.5 and 1:125) of the combined standard solution were prepared in mobile phase 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 an ondansetron and diphenhydramine mixture in 0.9% sodium chloride injection. Stability studies of the mixture would require an assay procedure that would detect and quantitate each analyte with reasonable accuracy and precision.

There were no reports in the scientific literature describing a separation of ondansetron and diphenhydramine hydrochlorides in a single injection. To develop a single isocratic HPLC method for these two analytes, our investigation indicated that chromatographic separation of the two compounds was best performed on the octadecylsilane column with a 60:40 v/v 0.1 M aqueous phosphate buffer pH 4.5 - acetonitrile mobile phase.

It was found that the ionic strength of the mobile phase was the

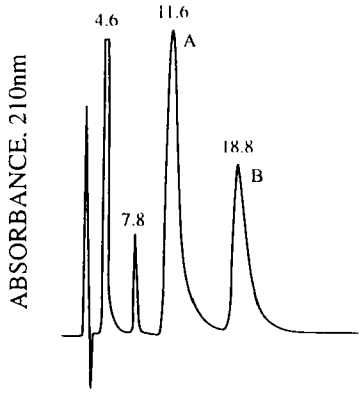


Figure 2. Typical HPLC chromatogram of ondansetron (A) and diphenhydramine (B) on an octadecylsilane column with acetonitrile - aqueous phosphate buffer pH 4.5 mobile phase. The peak at 4.6 min. retention time is methyl paraben and 7.8 min. is propyl paraben. See Experimental Section for assay conditions.

predominant parameter affecting retention of the analytes. Increasing the ionic strength significantly decreased retention times. Since the retention time of the analytes was also affected by the acetonitrile composition, 40% acetonitrile in the mobile phase offered the best separation of ondansetron and diphenhydramine hydrochloride in the shortest run time with no interference from the preservatives methyl paraben and propyl paraben commonly found in some commercial injections. A typical chromatogram of the two analytes is shown in Figure 2.

The HPLC method showed concentration versus absorbance linearity for ondansetron and diphenhydramine hydrochlorides in the 0.40 - 6.4 and 5.0 - 80.0 μg/mL ranges, respectively, at 210 nm. Table 1 gives other analytical figures of merit for each analyte.

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 the two analytes.

In summary, an octadecylsilane column with an aqueous 0.1 M phosphate buffer pH 4.5 - acetonitrile mobile phase has been shown to be amenable for the separation and the quantitation of an ondansetron - diphenhydramine

Table 1

Analytical Figures of Merit for Ondansetron and Diphenhydramine

Analyte	r^{2a}	System Suitability[b]	LOD[c] ng/mL	k'	Theoretical Plates[d]	Tailing Factor[c]	Rs
Ondan-setron	0.9994	1.8	70	3.7	1767	1.1	2.7
Diphen-hydramine	0.9999	1.3	105	6.6	2209	1.2	

[a]Range examined from 0.40 - 6.40 μg/mL ondansetron (n = 6) and 5.0 - 80.0 μg/mL diphenhydramine (n = 6). Mobile phase consisted of 60:40 v/v 0.1M phosphate buffer pH 4.5 - acetonitrile at 1.2 mL/min. with detection at 210 nm; [b]RSD % of 5 replicate injections at 0.80 μ/mL ondansetron and 10.0 μg/mL diphenhydramine at 210 nm; [c]Limit of detection, S/N = 3; [d]Calculated as N = 16 $(t/w)^2$; [e]Calculated at 5% peak height.

Table 2

Intra- and Inter-day Accuracy and Precision for Analysis of an Ondansetron (OND) and Diphenhydramine (DPH) Mixture

Concentration Added (μg/mL)	Concentration Found[a] (μg/mL)	RSD (%)	Error (%)
OND Intra-day[b]			
0.80	0.81 ± 0.01	1.8	1.0
5.12	5.04± 0.09	1.8	1.6
Inter-day[c]			
0.80	0.79 ± 0.03	3.8	1.2
5.12	5.06 ± 0.14	2.8	1.2
DPH Intra-day[b]			
10.0	9.80 ± 0.14	1.4	2.0
64.0	63.53 ± 1.10	1.7	0.7
Inter-day[c]			
10.0	9.62 ± 0.19	2.0	3.8
64.0	63.80 ± 1.70	2.7	0.3

[a]Mean ± std. dev. based on n =3; [b]n = 5; [c]n = 25.

hydrochlorides mixture in 0.9% sodium chloride injection. The method is free of interference from methyl and propyl parabens. This study suggests that the HPLC method can be used to investigate the chemical stability of the two drugs in sodium chloride injection.

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