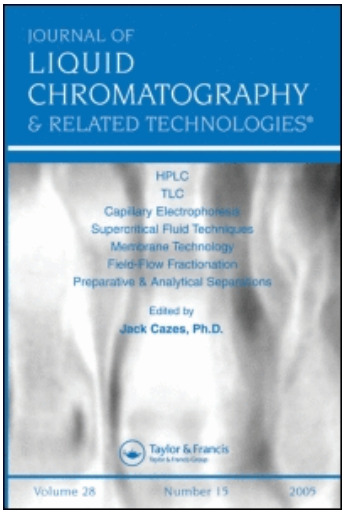

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HPLC DETERMINATION OF ONDANSETRON-ATROPINE AND ONDANSETRON-GLYCOPYRROLATE MIXTURES IN 0.9% SODIUM CHLORIDE INJECTION

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ABSTRACT

High Performance Liquid Chromatography procedures have been developed for the assay of ondansetron-atropine and ondansetron-glycopyrrolate mixtures in 0.9% sodium chloride injection. The separation and quantitation of the ondansetron-atropine mix was achieved on an octylsilane column at ambient temperature using a mobile phase of 60:40 v/v 0.01 M phosphate buffer, pH 4-acetonitrile at a flow rate of 1.0 mL/min with detection of the analytes at 254 nm. The separation is achieved within 15 min. The method showed linearity for ondansetron and atropine in the 266-1332 and 28-138 μg/mL ranges, respectively. Accuracy and precision were in the 0.2-5.6% and 0.4-1.8% ranges, respectively, for both drugs. The limits of detection for ondansetron and atropine were 2.1 ng/mL and 8.6 μg/mL, respectively, based on a signal to noise ratio of 3 and a 20 μL injection. The separation and quantitation of the ondansetron-glycopyrrolate mix was achieved on an octylsilane column at ambient temperature using a mobile phase of 55:45 v/v 0.01 M phosphate buffer, pH 4- acetonitrile at a flow rate of 1.0 mL/min with detection of the analytes at 254 nm. The separation is achieved within 15 min. The method showed linearity for

ondansetron and glycopyrrolate in the 500-2000 and 50-200 μg/mL ranges, respectively. Accuracy and precision were in the 2.5-3.7% and 0.1-1.5% ranges, respectively, for both analytes. The limits of detection for ondansetron and glycopyrrolate were 90 ng/mL and 6.9 μg/mL, respectively, based on a signal to noise ratio of 3 and a 20 μL injection.

INTRODUCTION

Mixtures containing ondansetron-atropine and ondansetron-glycopyrrolate are used as perioperative medications in operating room in U.S. hospitals. Interest in our laboratories in the stability and compatibility of these drug mixtures over time in 0.9% sodium chloride injection required the development of HPLC methods for each mixture. A search of the literature indicated that HPLC methods were not available to assay for the mixtures concurrently in a single injection.

Individually, atropine and glycopyrrolate have been previously analyzed by non-aqueous titrimetry (1,2), spectrophotometry (3), radioimmunoassay (4), and gas (5) and high performance liquid chromatography (2,6). The titrimetric assays were used to assay each respective drug substance in USP 23/NF 18. The spectrophotometric method measured glycopyrrolate in commercial tablets using a bromocresol purple-drug ion pair extracted into chloroform. The radioimmunoassay procedure was used to analyze as low as 2.5 ng/mL levels of atropine in plasma or serum. The GC method provided separation of atropine on a 3% loaded stationary phase in a glass column with flame ionization detection. One HPLC method for atropine injection used an octadecylsilane column with an acetonitrile-acetate buffer

containing tetrabutylammonium hydroxide as an ion-pair reagent. An HPLC method for glycopyrrolate was also reported that utilized an octadecylsilane column and an acetonitrile-methanol-water mobile phase containing sodium 1-pentanesulfonate as an ion-pair reagent.

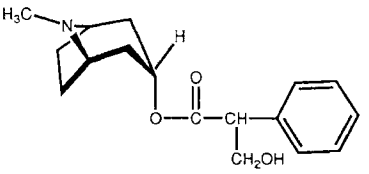
Ondansetron has been assayed by high performance thin-layer chromatography (HPTLC) and HPLC methods (7-9). The HPTLC method was developed especially for plasma samples, but the sample throughput was low and the equipment is not generally available in most laboratories. The HPLC assays used either a silica column with an aqueous-organic mobile phase or a cyanopropyl column operated in the reverse-phase mode.

In this paper, isocratic HPLC assays are presented that will simultaneously analyze for ondansetron-atropine or ondansetron-glycopyrrolate mixtures in 0.9% sodium chloride using a single injection. Each mixture is separated on an octylsilane column using a buffered aqueous-acetonitrile eluent. The separations are achieved within 15 min at ambient temperature with sensitivities in the ng/mL and μg/mL ranges for ondansetron and atropine/glycopyrrolate, respectively.

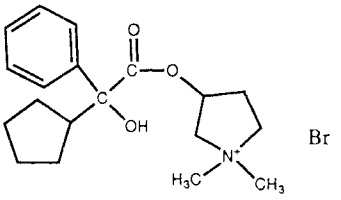
EXPERIMENTAL

Reagents and Chemicals

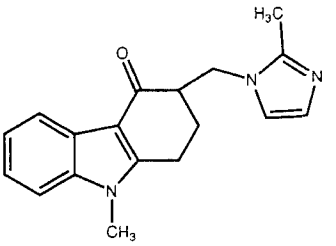
The structure formulae of the compounds studied are shown in *Figure* 1. Atropine sulfate (Lot K-1) and glycopyrrolate (Lot F-1) were



Atropine



Glycopyrrolate



Ondansetron

Figure 1 - Chemical Structures of Compounds Studied.

purchased from the United States Pharmacopeial Convention (Rockville, MD 20852). Ondansetron hydrochloride (Lots AWS17 or AWS332A) was a gift from Glaxo, Inc. (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 potassium phosphate and potassium hydroxide were J.T. Baker analyzed reagents (Phillipsburg, NJ 08865).

Instrumentation

The chromatographic separations were 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). The ondansetron-atropine separation was accomplished on an octylsilane column (RP-8 Spheri 5, 100 x 4.6 mm id, Applied Biosystems, Foster City, CA 94404). The mobile phase consisted of 60:40 v/v 0.01 M aqueous monobasic potassium phosphate, pH 4.0 (adjusted with 1 N potassium hydroxide)-acetonitrile.

The ondansetron-glycopyrrolate separation was achieved on an octylsilane column (RP-8 Spheri 5, 100 x 4.6 mm id, Applied Biosystems, Foster City, CA 94404). The mobile phase consisted of 55:45 v/v 0.01 M aqueous monobasic potassium phosphate, pH 4.0 (adjusted with 1 N potassium hydroxide)-acetonitrile.

Each 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 on both columns was 1 mL/min and the detector was set at 254 nm for both assays.

Preparation of Standard Solutions

A combined standard solution for ondansetron-atropine was prepared by adding accurately weighed samples of 14.8 mg of ondansetron hydrochloride and 1.65 mg of atropine sulfate into a 10 mL volumetric flask, and adding 0.9% sodium chloride injection to volume. This combined standard solution along with appropriate dilutions of the solution gave solutions containing 266, 666 and 1332 μg/mL of ondansetron and 27.6, 69.1 and 138.2 μg/mL of atropine expressed as free base concentrations. The same process was used to prepare a combined standard solution and dilutions for ondansetron-glycopyrrolate to obtain solutions containing 500, 1000 and 2000 μg/mL for ondansetron and 50, 100 and 200 μg/mL for glycopyrrolate expressed as free base concentrations. Three point calibration curves were constructed for each analyte in their respective mixtures and additional dilutions in sodium chloride injection were prepared to serve as spiked samples to determine accuracy and precision of method for each of the analytes. 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 HPLC assays using isocratic conditions for the analysis of an ondansetron-atropine or ondansetron-glycopyrrolate mixture in 0.9% sodium chloride injection. 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 separation and quantitation of ondansetron-atropine or ondansetron-glycopyrrolate in a single mixture. Studies to develop a single isocratic HPLC method for each mixture involved the use of underivatized silica, phenyl, octyl, base deactivated octyl and octadecyl columns with various mobile phases containing methanol-aqueous phosphate buffers and/or acetonitrile-aqueous phosphate buffers at 1 mL/min.

The octylsilane column was finally selected for both assays based upon peak separation and run-time parameters versus the actual concentration levels of the analytes in the injection stability study. In addition, there were no interferences from the paraben preservatives contained in the commercial injections. Typical chromatograms showing the separation of the two analytes in each of the two mixtures are shown in Figures 2 and 3. The detection wavelength of 254 nm was selected for both assays since it provided good accuracy and precision data for each two component mix.

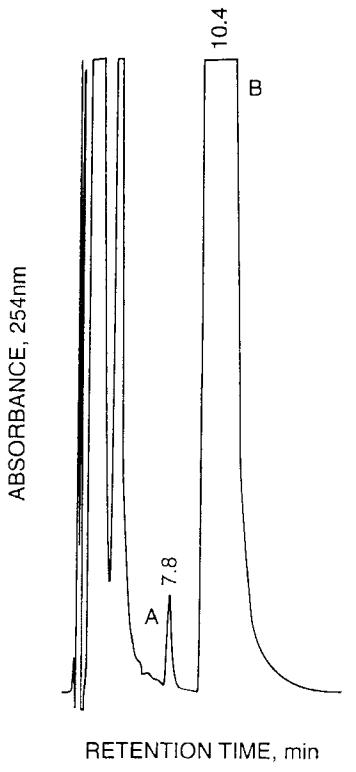


Figure 2 - Typical HPLC chromatogram of atropine (A) and ondansetron (B) on an octylsilane column with an aqueous phosphate buffer pH 4.0 - acetonitrile mobile phase. See Experimental Section for assay conditions.

The HPLC methods showed concentration versus absorbance linearity for the analytes at 254 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 the analytes would interfere with

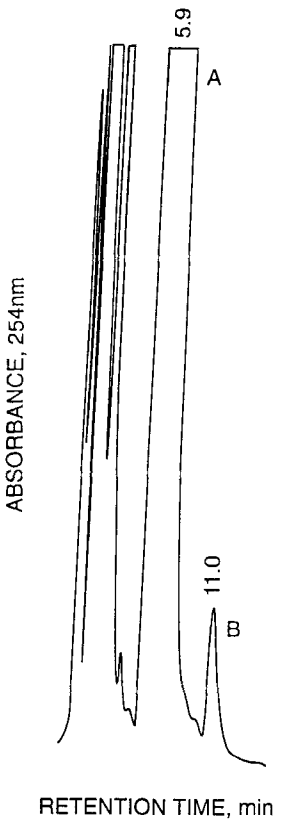


Figure 3 **Typical HPLC chromatogram of ondansetron (A) and glycopyrrolate (B) on an octylsilane column with an aqueous phosphate buffer pH 4.0- acetonitrile mobile phase. See Experimental Section for assay conditions.**

the quantitation of the other drug in the mixture at 254 nm. These experiments were performed on solutions of each drug in 0.9% sodium chloride injection after they had been degraded for 6 hr at 80 ± 1°C in both 1.0 N acid and 1.0 N base.

TABLE 1

Analytical Figures of Merit for Atropine, Glycopyrrolate and Ondansetron in Their Respective Mixtures.

Mixture	r^2	System Suitability	LOD[i]	k[l]	Theoretical Plates[j]	Tailing Factor[k]	Rs
1. Atropine	0.9988[a]	2.26[e]	8.6 μg/mL	4.91	915	1.4	3.4
Ondansetron	0.9939[b]	0.98[f]	2.1 ng/mL	6.99	984	1.8	
2. Glycopyrrolate	0.9989[c]	1.62[g]	6.9 μg/mL	7.94	1060	2.0	3.4
Ondansetron	0.9984[d]	1.62[h]	89.8 ng/mL	3.85	355	1.4	

[a] Range examined from 27.6 - 138.2 μg/mL atropine (n = 9).
[b] Range examined from 266.4 - 1332 μg/mL ondansetron (n = 9).
[c] Range examined from 50 - 200 μg/mL glycopyrrolate (n = 9).
[d] Range examined from 500.0 - 2000 μg/mL ondansetron (n = 9).
[e] Mean RSD% of 6 replicate injections at 69.1 μg/mL atropine,
[f] Mean % RSD of 6 replicate injections at 666 μg/mL ondansetron,
[g] Mean % RSD of 6 replicate injections at 100 μg/mL glycopyrrolate,
[h] Mean % RSD of 6 replicate injections at 1000 μg/mL ondansetron.
[i] Limit of detection, S/N = 3.
[j] Calculated as $N = 16 (t/w)^2$.
[k] Calculated at 5% peak height.

Table 2

Accuracy and Precision Using Samples With Added Drug

Analyte	Concn Added (μg/mL)	Concn Found (μg/mL)[a]	Percent Error	RSD (%)
1. Atropine	46.1	48.7 ± 0.87	5.6	1.8
	82.9	82.6 ± 0.78	0.4	0.9
Ondansetron	444.0	440.9 ± 5.21	0.7	1.2
	799.2	797.9 ± 3.52	0.2	0.4
2. Glycopyrrolate	77.3	79.2 ± 0.74	2.5	0.9
	154.6	160.3 ± 2.36	3.7	1.5
Ondansetron	750.0	776.3 ± 3.41	3.5	0.4
	1500.0	1538.4 ± 1.86	2.6	0.1

[a] Based on n = 3.

Percent error and precision of each method were evaluated using samples containing added analyte. The data shown in Table 2 indicate that both procedures give acceptable accuracy and precision for each analyte in each of the two mixtures.

Intra and inter-day variabilities of the assay for the ondansetron and atropine mixture were 2.14 (n = 6) and 0.98% (n = 15 over 3 days) and 3.33 (n = 6) and 2.26 (n = 15 over 3 days), respectively. Intra- and interday variabilities of the method for the ondansetron and glycopyrrolate mixture were 1.62 (n = 6) and 0.98% (n = 18 over 3 days) and 1.62 (n = 6) and 0.90% (n = 18 over 3 days), respectively.

In summary, an octylsilane column with an 60:40 v/v aqueous phosphate buffer pH 4- acetonitrile mobile phase for the ondansetron-

atropine assay and a 55:45 v/v aqueous phosphate buffer pH 4-acetonitrile mobile phase for the ondansetron-glycopyrrolate assay has been shown to be amenable for the separation and quantitation of each mixture in 0.9% sodium chloride injection. This study suggests that each HPLC method can be used to investigate the chemical stability of each drug in the respective mixtures.

ACKNOWLEDGEMENTS

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