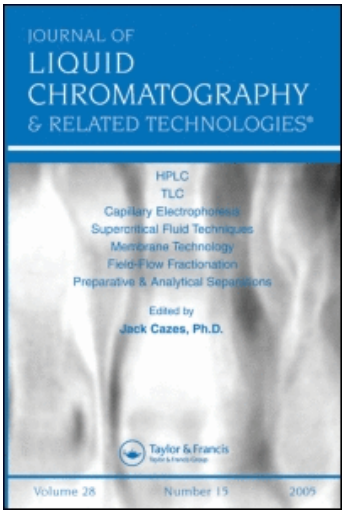

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HPLC Determination of a Metoclopramide and Ondansetron Mixture in 0.9% Sodium Chloride Injection

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HPLC DETERMINATION OF A METOCLOPRAMIDE 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 metoclopramide and ondansetron mixture in 0.9% sodium chloride injection. The separation and quantitation are achieved on a base deactivated octylsilane column at ambient temperature using a mobile phase of 77:23 v/v 0.01 M phosphate buffer, pH 4 - acetonitrile at a flow rate of 1.0 mL/min with detection of analytes at 273 nm. The separation is achieved within 15-20 min with sensitivity in the ng/mL range for each analyte. The method showed linearity for metoclopramide and ondansetron in the 12.5 - 50 and 5-20 μg/mL ranges, respectively. Accuracy and precision were in the 1 - 2% and 0.3 - 1.3% ranges, respectively, for both drugs. The limits of detection for metoclopramide and ondansetron were 49 and 20 ng/mL, respectively, based on a signal to noise ratio of 3 and a 20 μL injection.

INTRODUCTION

A mixture of metoclopramide and ondansetron can be used as a perioperative injection in operating rooms in U.S. hospitals. 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 the mixture concurrently in a single injection.

Metoclopramide has been previously analyzed by ultraviolet and fluorescence spectroscopy and gas-liquid and high performance liquid chromatography. The UV assay measured the drug at 305 nm in a chloroform extract of commercial tablets (1). The fluorescence method measured the drug in a pH 2 solution using excitation and emission wavelengths of 310 and 360 nm, respectively (2). The GC assay provided separation of the drug on a 6 ft 3% OV-1 stationary phase with flame ionization detection (3). One HPLC method reported the separation of metoclopramide on an octadecylsilane column with a methanol-water-ammonia mobile phase and detection at 308 nm (4). The most recent HPLC method used an acetate buffer-acetonitrile mobile phase to assay for the drug in commercial tablets with the UV detector set at 273 nm (5).

Ondansetron has been assayed by high performance thin-layer chromatography (HPTLC), HPLC methods and radioimmunoassay methods. 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 (6). The HPLC assays used either

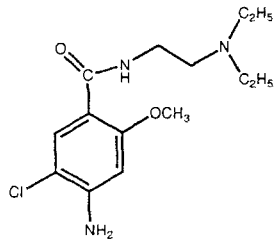
a silica column with an aqueous-organic mobile phase or a cyanopropyl column operated in the reverse-phase mode (7,8). Detection of the analyte was either by UV at 305 nm or radiochemical detection. The radioimmunoassay was combined with sample cleanup using a cyanopropyl solid phase extraction cartridge to provide a subnanogram per mL determination of ondansetron (9).

In this paper, an isocratic HPLC assay is presented that will simultaneously analyze for metoclopramide and ondansetron in 0.9% sodium chloride using a single injection. The compounds are separated on a base deactivated octylsilane column using a buffered aqueous-acetonitrile eluent. The separation is achieved within 15-20 min at ambient temperature with sensitivity in the ng/mL range.

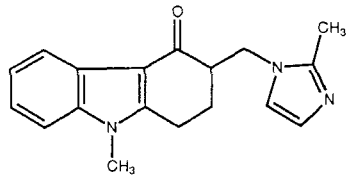
EXPERIMENTAL

Reagents and Chemicals

The structure formulae of the compounds studied are shown in Figure 1. Metoclopramide hydrochloride (Lot 75F-0603) was purchased from Sigma Chemical Co. (St. Louis, MO). 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 Baker analyzed reagents.



METOCLOPRAMIDE



ONDANSETRON

Figure 1 Chemical structures of compounds studied.

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 25 cm base deactivated octylsilane (4.6 mm i.d., 5 μm particle size, Zorbax Rx-C8 Mac-Mod Analytical, Chadds Ford, PA 19317). The mobile phase consisted of 77:23 v/v 0.01M aqueous monobasic potassium phosphate,

pH 4.0 (adjusted with 1 N potassium 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 1 mL/min and the detector was set at 273 nm.

Preparation of Standard Solutions

A combined standard solution containing metoclopramide and ondansetron was prepared by accurately weighing 2.8 mg of metoclopramide hydrochloride and 1.1 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 1:1 and 1:4 dilutions made from the combined standard solution gave solutions containing 50.0, 25.0 and 12.5 μg/mL of metoclopramide and 20.0, 10.0, and 5.0 μg/mL of ondansetron expressed as the free base concentrations. Three point calibration curves were constructed for each analyte. Additional dilutions (7.5:10 and 3.8:10) 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

There were no reports in the scientific literature describing a separation of metoclopramide and ondansetron in a single mixture. Initial

studies to develop a single isocratic HPLC method for the two analytes involved the use of underivatized silica, phenyl, octyl, and octadecyl columns with various mobile phases containing methanol-aqueous phosphate buffers and/or acetonitrile-aqueous phosphate buffers at 1 mL/min. The best resolution of the analytes was obtained on a base deactivated octylsilane column using a 77:23 v/v phosphate buffer pH 4-acetonitrile mobile phase with a total run time of 15-20 min. The column also allowed the separation of methylparaben (preservative found in most commercial injections) from the analytes (Rt of 17.8 min). A typical chromatogram showing the separation of the two analytes is shown in Figure 2.

The absorption maximum for metoclopramide in the phosphate bufer-acetonitrile mobile phase was 273 nm. Even though this was not the maximum absorption wavelength for ondansetron, 273 nm was selected as the detection wavelength for the assay since it provided both good accuracy and precision data for the two component mix.

The HPLC method showed concentration versus absorbance linearity for metoclopramide and ondansetron in the 12.5-50 and 5-20 μg/mL ranges, respectively, at 273 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 the quantitation of each drug at 273 nm. These experiments were performed on solutions of both drugs in 0.9% sodium chloride injection

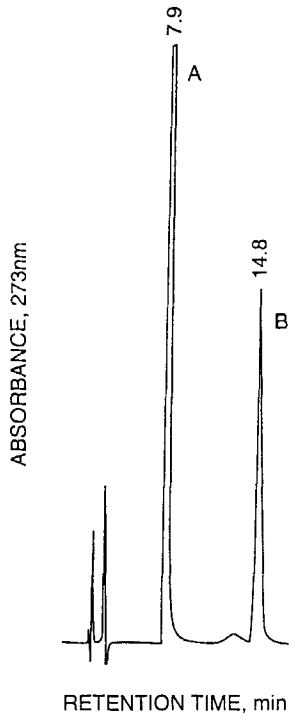


Figure 2 Typical HPLC chromatogram of metoclopramide (A) and ondansetron (B) on a base deactivated octylsilane with an aqueous phosphate buffer pH 4.0 - acetonitrile mobile phase. See Experimental Section for assay conditions.

after they had been degraded for 6 hr at 80°C in both 1.0N hydrochloric acid and 1.0N sodium hydroxide.

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 both analytes.

Table 1

Analytical Figures of Merit for Metoclopramide and Ondansetron

Analyte	r^{2a}	System Suitability[b]	LOD[c] ng/mL	k′	Theoretical Plates[d]	Tailing Factor[e]	Rs
Metoclopramide	0.9994	0.75	49.0	1.78	4456	1.5	
							9.4
Ondansetron	0.9992	0.43	20.0	3.93	4577	2.0	

[a] Range examined from 12.5-50 μg/mL metoclopramide (n = 9) and 5.0 - 20 μg/mL ondansetron (n = 9). Mobile phase consisted of 77:23 v/v 0.01M phosphate buffer, pH 4-acetonitrile at 1.0 mL/min with detection at 273 nm.
[b] Mean RSD% of 6 replicate injections at 25.0 μg/mL metoclopramide and 10.0 μg/mL ondansetron at 273 nm.
[c] Limit of detection, S/N = 3.
[d] Calculated as $N = 16\ (tr/w)^2$
[e] Calculated at 10% peak height

Table 2

Accuracy and Precision Using Spiked Drug Samples

	Concn Added (μg/mL)	Concn Found* (μg/mL)	Percent Error	RSD (%)
Metoclopramide	37.65	38.10 ± 0.12	1.20	0.31
	18.83	19.08 ± 0.25	1.33	1.31
Ondansetron	14.87	15.07 ± 0.04	1.34	0.27
	7.44	7.56 ± 0.05	1.61	0.66

* Based on n = 3.

Intra-day variabilities of the assay for metoclopramide and ondansetron expressed as % RSD were 0.75 and 0.43% (n = 6), respectively. Inter-day variabilities of the assay for these drugs were 2.2 and 2.4% (n = 18 over 3 days), respectively.

In summary, a base deactivated octylsilane column with an aqueous 0.01 M pH 4 buffer-acetonitrile mobile phase has been shown to be amenable for the separation and quantitation of a metoclopramide-ondansetron mixture in 0.9% sodium chloride injection. This study suggests that the HPLC method can be used to investigate the chemical stability of a mixture of the drugs in sodium chloride injection.

ACKNOWLEDGEMENTS

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