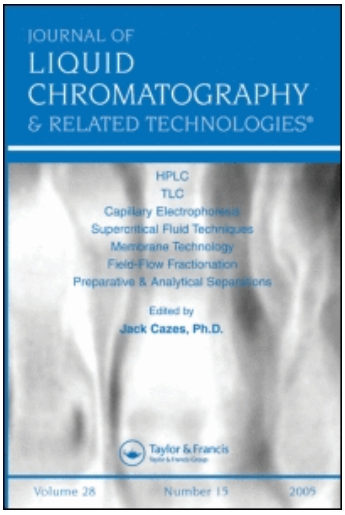

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HPLC DETERMINATION OF MORPHINE-ONDANSETRON AND MEPERIDINE-ONDANSETRON MIXTURES IN 0.9% SODIUM CHLORIDE INJECTION

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ABSTRACT

High performance liquid chromatography procedures have been developed for the assay of morphine-ondansetron and meperidine-ondansetron mixtures in 0.9% sodium chloride injection. The separation and quantitation of morphine-ondansetron were performed on an underviatized silica column at ambient temperature using a mobile phase of 60:40 v/v 0.01 M monobasic potassium phosphate pH 4.0 - methanol at a flow rate of 1.0 mL/min with the detection set at 233 nm. The separation was achieved within 20 min. Morphine and ondansetron were linear in the 134 - 536 and 68 - 271 μg/mL ranges, respectivly. Accuracy and precision were in the range 0.04 - 4 and 0.2 - 1%, respectively, for the two analytes and the limits of detection for morphine and ondansetron were 210 and 110 ng/mL, respectively, based on a signal to noise ratio of 3 and a 20 μL injection. The separation and quantitation of the meperidine-ondansetron mixture were also achieved on an underivatized silica column at ambient temperature using a mobile

phase of 60:40 v/v 0.01 M monobasic potassium phosphate pH 4.0 - methanol at a flow rate of 1.0 mL/min with detection of the two analytes at 254 nm. The separation was achieved within 20 min. Meperidine and ondansetron were linear in the 556 - 3331 and 89 - 536 μg/mL ranges, respectively. Accuracy and precision were in the range 0.7 - 5.1 and 0.1 - 0.6%, respectively, for the two analytes and the limits of detection for meperidine and ondansetron were 1.73 μg/mL and 47 ng/mL, respectively, based on a signal to noise ratio of 3 and a 20 μL injection.

INTRODUCTION

Mixtures of morphine-ondansetron (Mixture A) and meperidine-ondansetron (Mixture B) are administered as perioperative injections in U.S. hospitals. Interest in our laboratories in the stability and compatibility of each drug mixture over time in 0.9% sodium chloride injection required the development of HPLC methods. A search of the literature indicated that HPLC methods were not available to assay each analyte in mixture A or mixture B concurrently in a single injection.

Morphine has been analysed by HPLC with electrochemical (EDC) or UV detection.[1-3] The HPLC methods are not as sensitive as radioimmunoassay procedures, but are more specific and often used in the analysis of the compound. The HPLC-ECD method involved separation of morphine on an octylsilane column using a mobile phase of 15:85 v/v absolute methanol-50 mM dibasic sodium phosphate pH 3.5 containing 3 mM octanesulphonic acid.[1] The electrode potential was set at +600 mV vs Ag/AgCl. Morphine has also been analysed using UV detection at 210 nm with an HPLC system consisting of an octadecylsilane column operating at a flow rate of 0.8 mL/min and 26.5:73.5 v/v acetonitrile - 0.8mM sodium dodecyl sulphate in 10 mM monobasic phosphate buffer mobile phase.[3] The official USP 23 assay for morphine utilizes an HPLC separation on an octadecylsilane column with detection at 284 nm.[4]

Meperidine has been assayed by a variety of analytical methods. Gas chromatography using mass spectrometry has been reported.[5,6] Spectrophotometry, colorimetry and derivative spectroscopy have also been utilized by various investigators.[7-9] The use of an ion-selective electrode was reported by one laboratory.[10] The official USP 23 assays for drug substance and a syrup dosage form utilize a reverse-phase HPLC separation and non-aqueous titrimetry, respectively.[11,12]

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.[13] The HPLC assays used either a silica column with an aqueous-organic mobile phase or a cyanopropyl column operated in the reverse phase mode.[14,15] 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.[16]

In this paper, isocratic HPLC assays are presented for the simultaneous analysis of morphine and ondansetron (Mixture A) and meperidine and ondansetron (Mixture B) in 0.9% sodium chloride injection. Both mixtures were separated on an underivatized silica column using a buffered aqueous-methanol eluent. Each separation was achieved within 20 min with sensitivity in the μg/mL range for meperidine and ng/mL for morphine and ondansetron.

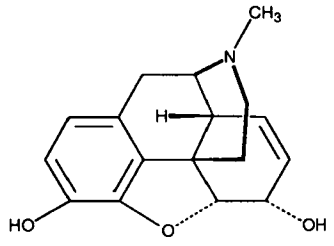
EXPERIMENTAL

Reagents and Chemicals

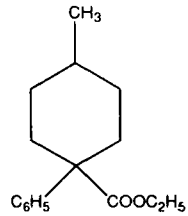
The structural formulae of the compounds studied are shown in Figure 1. Morphine sulfate and meperidine hydrochloride were purchased as their respective salts from The United States Pharmacopeia (Rockville, MD 20852, Lots H-1 and G-1, respectively). Ondansetron hydrochloride (Lot No. AWS332A) was a gift from Glaxo, Inc. (Research Triangle Park, NC 27709). Methanol (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, potassium hydroxide and concentrated phosphoric acid were Baker analysed reagents.

Instrumentation

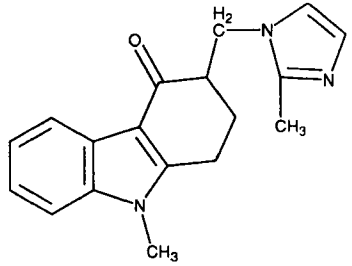
The chromatographic separations were performed on an HPLC system consisting of a Waters Model 501 pump (Milford, MA 01757), an Alcott Model 728 autosampler (Norcross, Ga 30093) equipped with a 20 μL loop, a Beckman Model 163 variable wavelength UV-VIS detector (Fullerton, CA 92634) and a



MORPHINE



MEPERIDINE



ONDANSETRON

Figure 1. Chemical stuctures of compounds studied.

Hewlett Packard Model 3395 integrator (Palo Alto, CA). Separation of Mixture A was achieved on a 22 cm underivatized silica column (4.6 mm i.d., 5 μm particle size, Brownlee Silica Applied Biosystems, Inc. San Jose, CA 95134). The mobile phase consisted of 60:40 v/v 0.01 M aqueous monobasic potassium phosphate, pH 4.0 (adjusted with 10% phosphoric acid)-methanol. The separation of Mixture B was also accomplished on a 22 cm underivatized silica column. The mobile phase consisted of 60:40 v/v 0.01 M aqueous monobasic potassium phosphate pH 4.0 (adjusted with 10% phosphoric acid)-methanol. The mobile phases were 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 1.0 mL/min for both mixtures and the detector was set at 233 nm for mixture A and 254 nm for mixture B.

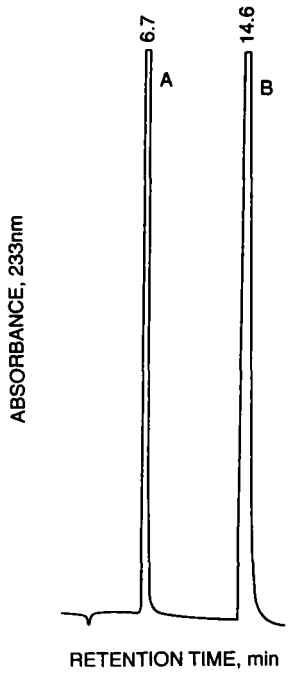


Figure 2. Typical HPLC chromatogram of Morphine (A) and Ondansetron (B) on a silica column with 60:40 v/v 0.01 M phosphate buffer pH 4.0 - methanol. See Experimental Section for assay conditions.

Preparation of Standard Solutions

A combined standard solution containing morphine and ondansetron was prepared by accurately weighing 35.5 mg of morphine sulfate and 15.0 mg ondansetron hydrochloride in a 10 mL volumetric flask. Another standard solution containing meperidine and ondansetron was prepared by accurately weighing 95.6 mg of meperidine hydrochloride and 15.0 mg ondansetron hydrochloride in a 10 mL volumetric flask. Sodium chloride injection was added to each mixture and the flasks were shaken vigorously for 2 minutes followed by addition of 0.9% sodium chloride to volume. Dilutions 1:5, 1:7.5 and 1:20 of the combined morphine-ondansetron standard solution and 1:2.5, 1:7.5 and 1:15 dilutions of the combined meperidine-ondansetron standard solution gave solutions in the 134-536 μg/mL and 68 - 271 μg/mL range for morphine-ondansetron, respectively, expressed as free base concentrations and 555-3331 μg/mL and 89-536 μg/mL range for

Table 1

Analytical Figures of Merit for Morphine-Ondansetron and Meperidine-Ondansetron Mixtures

Mixture	r^{2a}	System Suitability[b]	LOD ng/ml[c]	k^1	Theoretical Plates[d]	Tailing Factor[e]	Rs
A							
Morphine	0.9994	0.57	210	1.6	3000	1.1	
Ondansetron	0.9991	0.79	110	4.7	3779	1.2	11.5
B							
Meperidine	0.9969	0.98	1730	2.4	2969	2.5	
Ondansetron	0.9998	1.04	47	4.6	5978	2.0	8.0

[a] Range examined from 134-536μg/mL morphine (n = 9) and 68-271 μg/mL ondansetron for Mixture A at 233 nm and 555-331 μg/mL meperidine and 89-536 μg/mL Ondansetron for Mixture B at 254 nm.
[b] Mean RSD% of 6 replicate injections at 268 μg/mL morphine and 136 μg/mL ondansetron for Mixture A at 233 nm and 1110 μg/mL meperidine and 179 μg/mL ondansetron for Mixture B at 254 nm.
[c] Limit of Detection, S/N = 2.
[d] Calculated as $N = 16 (tr/w)^2$.
[e] Calculated at 5% peak height.

meperidine-ondansetron, respectively, expressed as free base concentrations. Dilutions (1:10 and 1:15 for Mixture A standard solutions and 1:5 and 1:10 for Mixture B standard solutions) were prepared in 0.9% sodium chloride injection to serve as spiked samples for each analyte to determine accuracy and precision. 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 separations of morphine-ondansetron and meperidine-ondansetron mixtures. Initial studies to develop HPLC methods for each mixture using isocratic conditions involved the use of underivatized silica, phenyl, octyl, deactivated octyl and octadecyl columns with various mobile phases containing methanol-aqueous phosphate buffers

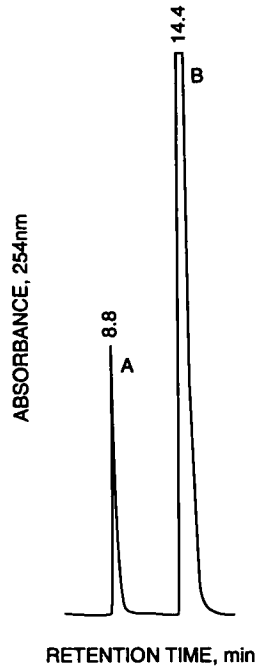


Figure 3. Typical HPLC chromatogram of Meperidine (A) and Ondansetron (B) on a silica column with 60:40 v/v 0.01 M phosphate buffer pH 4.0 - methanol. See Experimental Section for assay conditions.

and/or acetonitrile-aqueous phosphate buffers at 1 mL/min. The best resolution of the analytes in both Mixtures A and B was obtained on an underivatized silica column using a 60:40 v/v phosphate buffer pH 4-methanol mobile phase with total run times of 15-20 min. The column also allowed the separation of methylparaben (preservative found in commercial injections) from the analytes (R_t of 3 min). Typical chromatograms showing the separation of each mixture are shown in Figures 2 and 3.

From an earlier study in this lab, it was shown that morphine and ondansetron absorbed strongly at 233 nm in a methanol-phosphate buffer solvent system. It was also determined that meperidine and ondansetron absorb around 254 nm in the same solvent system. Therefore, 233 nm and 254 nm were selected as the

Table 2

Accuracy and Precision Using Samples with Added Drug

Mixture	Concn Added μg/mL	Concn Found μg/mL	Percent Error	RSD (%)
A				
Morphine	179	171.9 ± 1.7	4.0	1.0
	358	359.6 ± 0.6	0.4	0.2
Onansetron	91	88.6 ± 0.9	2.6	1.0
	181	187.6 ± 1.0	3.6	0.5
B				
Meperidine	833	845.9 ± 0.8	1.5	0.1
	1665	1750.8 ± 6.0	5.1	0.3
Ondansetron	134	134.9 ± 0.8	0.7	0.6
	268	278.0 ± 1.3	3.7	0.5

[a] Mean ± standard deviation based on n = 3.

detection wavelengths for morphine-ondansetron and meperidine-ondansetron mixtures, respectively, since they provided good accuracy and precision data for the two component mixtures.

The HPLC method for Mixture A showed concentration versus absorbance linearity for morphine-ondansetron in the 134-536 and 68 - 271 μg/mL ranges, respectively, at 233 nm. Table 1 gives the analytical figures of merit for each of the analytes in Mixture A. The HPLC method for Mixture B showed concentration versus absorbance linearity for meperidine-ondansetron in the 555-3331 and 89-536 μg/mL ranges, respectively, at 254 nm. Table 1 also gives the analytical figures of merit for each of the analytes in Mixture B. A photodiode array detector (Model 990, Waters Associates, Milford, MA 01757) was used to verity that none of the degradation products of the analytes in either Mixture A or B (analysed under their respective analytical conditions) interfered with the quantitation of each drug at 233 or 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°C in both 1.0 N hydrochloric acid and 1.0 N sodium hydroxide.

Percent error and precision of the methods were evaluated using spiked samples containing each analyte. The results for mixtures A and B are shown in Table 2. The results indicate that the procedures give acceptable accuracy and precision for the analytes in both mixtures.

Intra-day variabilities for morphine and ondansetron (Mixture A) expressed as % RSD were 0.57 and 0.79% (n=6), respectively. Inter-day variabilities of the assay for morphine and ondansetron were in the 0.45-0.79 and 0.51-1.03% (n=18 over 3 days) ranges, respectively. Intra-day variabilities for meperidine and ondansetron (Mixture B) expressed as % RSD were 0.98 and 1.04% (n=6), respectively. Inter-day variabilities of the assay for meperidine-ondansetron were in the 0.98-1.49 and 0.48-1.92% (n=18 over 3 days) ranges, respectively.

In summary, an underivatized silica column with an aqueous 0.01 M pH 4.0 buffer-methanol mobile phase was shown to be suitable for the separation and quantitation of a morphine-ondansetron mixture (A) and a meperidine-ondansetron mixture (B) in 0.9% sodium chloride injection. This study suggests that the above listed HPLC methods can be used to investigate the chemical stability of the analytes in either mixture.

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