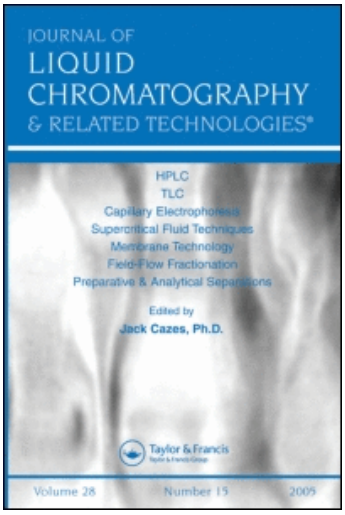

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HPLC DETERMINATIONS OF ONDANSETRON WITH SELECTED MEDICATIONS IN 0.9% SODIUM CHLORIDE INJECTION USP

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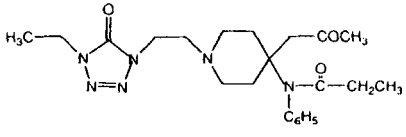
ABSTRACT

High performance liquid chromatography procedures have been developed for the assay of ondansetron-selected medications in 0.9% sodium chloride injection USP. The separation and quantitation of each mixture were performed on either an underivatized silica column or a bonded phase column at ambient temperature using either a methanol or acetonitrile-phosphate buffer mobile phase in the 4-5.4 pH ranges with detection set at 254, 233 or 210 nm. The separations were usually achieved within 20 min. Linearity, limit of detection, retention and tailing factors, resolution, accuracy and precision were calculated for each mixture.

INTRODUCTION

Mixtures of ondansetron with neostigmine, naloxone, droperidol, midazolam, ranitidine, fentanyl or alfentanyl are used as perioperative injections in operating rooms in U.S. hospitals. Interest in our laboratories, in



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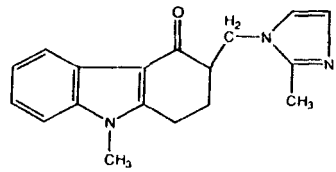

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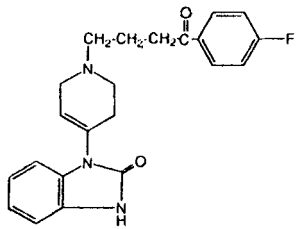

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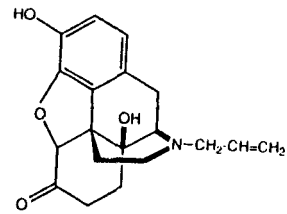

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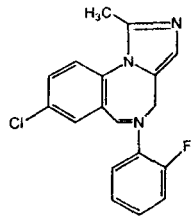

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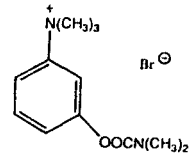

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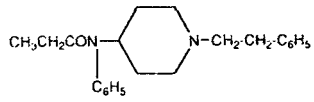

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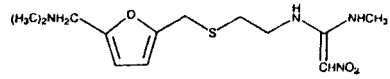

RANITIDINE

Figure 1. Chemical Structures of Compounds Studied.

stability and compatibility of these drug mixtures over time in 0.9% sodium chloride injection USP, required the development of HPLC methods. A search of the literature indicated that no HPLC methods were available to assay for these ondansetron mixtures in a single injection.

Neostigmine has been analyzed by gas chromatography (GC)[1], non-aqueous titrimetry[2,3] high performance liquid chromatography (HPLC)[4] and thin layer chromatography-mass spectrometry (TLC-MS)[5]. Among the methods for naloxone assay are non-aqueous titrimetry[6], HPLC with ultraviolet, electrochemical and coulometric detection[7-9] and radioimmunoassay (RIA)[10]. Procedures reported for droperidol include non-aqueous titrimetry[11], HPLC[12], colorimetry,[13] UV spectrophotometry,[14] and GC.[14] Midazolam has been analyzed by UV spectrophotometry,[15] LC-MS,[16] GC,[17] HPLC[18] and TLC coupled with fast atom bombardment MS.[19] Among the methods reported for ranitidine are polarography,[20] spectrophotometry-colorimetry,[21,22] capillary electrophoresis (CE),[23] non-aqueous titrimetry,[24] and HPLC.[25] Fentanyl has been assayed by non-aqueous titrimetry,[26] HPLC,[27-29] GC-MS,[30] voltammetry,[31] and solid phase RIA.[32] Methods for alfentanyl feature HPLC with UV detection,[33] GC-MS,[34,35] capillary GC,[36] and RIA.[37] Ondansetron has been assayed by high performance thin layer chromatography (HPTLC),[38] HPCL[39] and RIA[40] methods.

In this paper, isocratic HPLC assays are presented that will similtaneously analyze for ondansetron with selected medications in 0.9% sodium chloride injection using a single injection. The analytes are separated on silica or bonded phase columns using either methanol or acetonitrile-aqueous phosphate buffer mobile phases pH 4-5.4. The separations are achieved within 20 min at ambient temperature with sensitivity in the ng/mL range for all analytes except neostigmine, where sensitivity was in the μg/mL range.

EXPERIMENTAL

Reagents and Chemicals

The structural formulae of the medications studied are shown in Figure 1. Neostigmine bromide and fentanyl citrate were purchased as their respective salts from The United States Pharmacopeia (Rockville, MD 20852, Lot G and H, respectively). Ondansetron hydrochloride (Lot AWS332A) and ranitidine hydrochloride (Lot AWS22C) were gifts from Glaxo, Inc. (Research Triangle Park, NC 27709). Naloxone hydrochloride (Lot 63H 0286) was purchased from Sigma Chemical Co. (St. Louis, MO 63178) and midazolam (Lot 823103) was a gift from Roche Pharmaceuticals (Nutley, NJ). Alfentanyl hydrochloride injection (Lot 53C015, exp date 9/95) was purchased from Janssen Pharmaceuticals (Titusville, NJ 08560) and droperidol (Lot V860-339) was secured from Research Diagnostics, Inc. (Flanders, NJ 07836). Methanol and

Table 1

HPLC Chromatographic Conditions for Ondansetron Mixtures

Mixt.	Component	Column	Vendor	Mobile Phase[a]	Detection nm	% RSD Intra- (n=6)	% RSD Inter- (n=18)[b]
1	Ondansetron Neostigmine	Phenyl 300x3.9 mm i.d., 10μm	μ-Bondapak Waters[2]	55 : 50 v/v 0.01M buffer[1] pH 5.4-Acetonitrile	254	0.52 2.90	2.70 3.93
2	Ondansetron Naloxone	Silica 220x4.6 mm i.d., 5μm	Brownlee Applied Biosystems[3]	60 : 40 v/v 0.01M buffer[1] pH 4.0-Methanol	254	0.50 0.40	0.55 1.23
3	Ondansetron Droperidol	Phenyl 300x4.6 mm i.c., 10μm	μ-Bondapak Waters[2]	50 : 50 V/V 0.01M buffer[1] pH 4.0-Aetonitrile	245	0.33 0.38	0.32 0.28
4	Ondansetrol Midazolam	Phenyl 150x4.6 mm i.d., 5μm	Zorbax SB MacMod Analytical[4]	50 : 50 v/v 0.01M buffer[1] pH 5.4-Acetonitrile	233	2.70 0.56	3.46 0.84
5	Ondansetron Rantidine	Silica 220x4.6 mm i.d., 5μm	Brownlee Applied Biosystems[3]	60 : 40 v/v 0.01M buffer[1] pH 4.0-Methanol	233	1.20 0.28	0.96 0.35
6	Ondansetron Fentanyl	Octylsilane 100x4.6 mm i.d., 5μm	Brownlee Applied Biosytems[3]	50 : 50 v/v 0.01M buffer[1] pH 4.0-Acetonitrile	210	0.30 1.44	0.34 1.37
7	Ondansetron Afentanyl	Phenyl 300x4.6 mm i.d., 10μm	μ-Bondapak Waters[2]	50 : 50 v/v 0.01M buffer[1] pH 5.4-Acetonitrile	210	0.71 2.20	0.59 1.40

[a] Flow rate for all separations was 1.0 mL/min
[b] Six (6) replicates per day for 3 days
[1] Aqueous monobasic potassium phosphate
[2] Milford, MA 01757
[3] San Jose, CA 95134
[4] Chadds Ford, PA 19317

acetonitrile (J.T. Baker, Phillipsburg, NJ 08865) were HPLC grade and water was purified by a cartridge system (Continental Water Systems, Roswell, Ga 30076). Monobasic potassium phosphate and concentrated phosphoric acid were Baker analyzed reagents.

Table 2

Analytical Figures of Merit for Ondansetron Mixtures

Mixture	Component(s)	Concn. Range Examined µg/mL	r^{2a}	System Suitability[b]	LOG	k	N[d]	Tailing Factor[e]	Rs
1	Ondansetron	26-131 µg/mL	0.9994	0.52	42	4.6	1167	1.3	5.0
	Neostigmine	3.5-17 µg/mL	0.9998	2.90	1690	2.8	1843	1.1	
2	Ondansetron	13-67 µg/mL	0.9995	0.50	42	4.2	2725	1.2	9.3
	Naloxone	1.3-6.6 µg/mL	0.9986	0.40	325	1.9	5449	1.0	
3	Ondansetron	5-20 µg/mL	0.9994	0.33	31	2.2	1609	1.0	2.5
	Droperidol	6.2-25µg/mL	0.9999	0.38	39	3.2	1564	1.1	
4	Ondansetron	0.2-2.0 µg/mL	0.9848	2.70	3	1.8	2558	1.5	1.9
	Midazolam	0.5-5.0 µg/mL	0.9999	0.56	63	2.2	3388	2.3	
5	Ondansetron	5-20 µg/mL	0.9944	1.20	98	4.9	4969	1.5	5.0
	Rantidine	60-250µg/mL	0.9943	0.28	20	3.1	1963	1.3	
6	Ondansetron	2.7-27 µg/mL	0.9999	0.30	5	6.3	1987	1.0	5.2
	Fentanyl	0.3-3.3 µg/mL	0.9999	1.44	21	10.7	1964	1.7	
7	Ondansetron	27-68 µg/mL	0.9999	0.71	53	5.2	1458	1.4	5.5
	Alfentanyl	0.9-3.6 µg/mL	0.9999	2.20	56	2.4	1444	1.2	

Instrumentation

The chromatograpic 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 Hewlett Packard Model 3395 integrator (Palo Alto, CA). The chromatographic conditions used for each mixture are shown in Table 1. The mobile phases were filtered through a 0.45 µm nylon 66 filter (MSI, Westborough, MA 01581) and degassed by sonication prior to use.

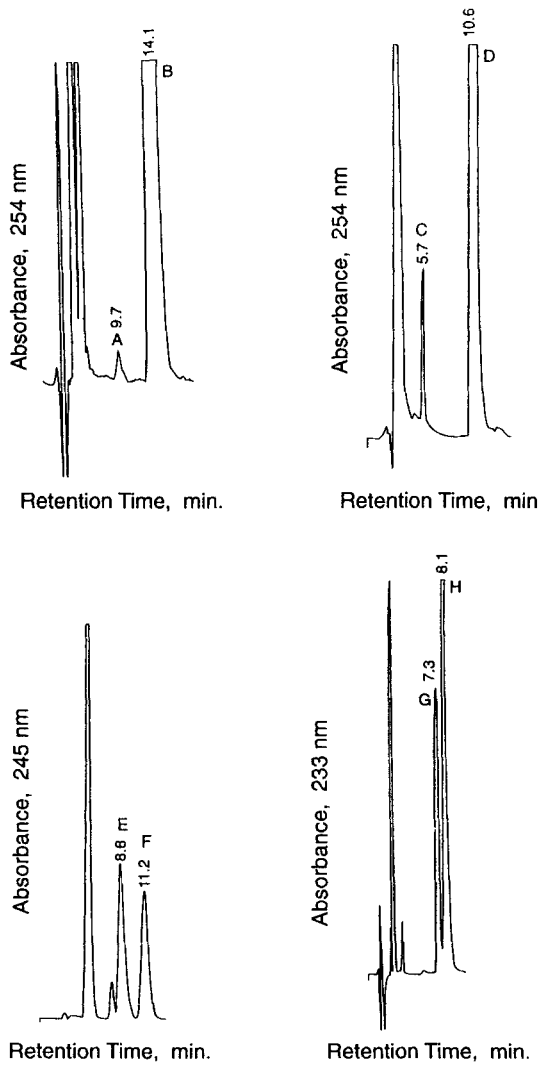

Figure 2. Top Left - Typical HPLC Chromatogram of neostigmine (A) and ondansetron (B) on a phenyl column with 55:50 v/v aqueous phosphate buffer pH 5.4-acetonitrile. Top right - Typical HPLC chromatogram of naloxone (C) and ondansetron (D) on a silica column with 60:40 v/v aqueous phosphate buffer pH 4.0-acetonitrile. Bottom Left - Typical HPLC chromatogram of ondansetron (E) and droperidol (F) on phenyl column with 50:50 v/v aqueous phosphate buffer pH 4-acetonitrile. Bottom right - Typical HPLC chromatogram of ondansetron (G) and midazolam (H) on a phenyl column with 50:50 v/v aqueous phosphate buffer pH 5.4-acetonitrile. See Table 1 for assay conditions.

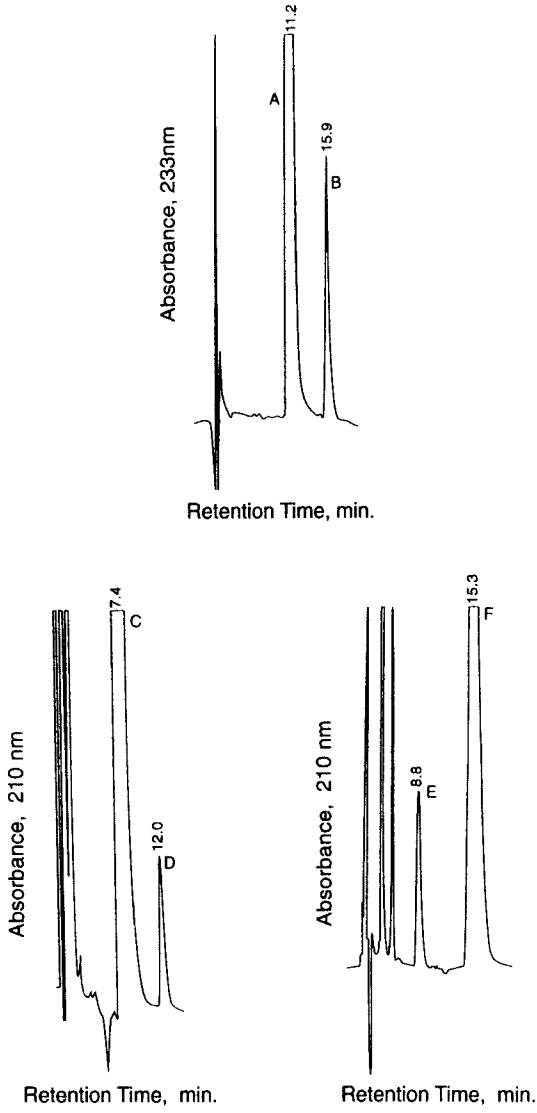

Figure 3. Top Center- Typical HPLC chromatogram of ranitidine (A) and ondansetron (B) on a silica column with 60:40 v/v aqueous phosphate buffer pH 4-methanol. Bottom Left - Typical HPLC chromatogram of ondansetron (C) and fentanyl (D) on an octylsilane column with 50:50 v/v aqueous phosphate buffer pH 4 - acetonitrile. Bottom right - Typical HPLC chromatogram of alfentanyl (E) and ondansetron (F) on a phenyl column with 50:50 v/v aqueous phosphate buffer pH 5.4 - acetonitrile. See Table 1 for assay conditions.

Preparation of Standard Solutions

Combined standard solutions containing ondansetron and the other medication in each mixture were prepared by accurately weighing the reference or working standard powder into an appropriately size volumetric flask and adding 0.9% sodium chloride injection USP to volume. For ondansetron-midazolam and ondansetron-droperidol mixtures, acetonitrile and the HPLC mobile phase were used to dissolve the weighed powders, respectively, for the standard solutions. Dilutions of each combined standard solution were prepared to achieve the concentration ranges (expressed as free base concentrations) shown in Table 2. Other dilutions within the concentration ranges examined were prepared 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 HPLC separations of mixtures of ondansetron with selected medications. 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 and/or acetonitrile-aqueous phosphate buffers at 1 mL/min. The mobile phase and column giving the best resolution for a given mixture were then selected for the assay. The columns also allowed the separation of methylparaben (preservative found in many commercial injections) from the analytes. Typical chromatograms showing the separation of each of the seven drug mixtures are shown in Figures 2 and 3.

Earlier studies in this lab had demonstrated that more than one wavelength might be appropriate for detection of each drug mixture. The final selection of a suitable detection wavelength was based on sensitivity needs of the assay as well as accuracy and precision data for each two component mixture.

The HPLC methods showed concentration versus absorbance linearity for each mixture in the concentration range studied (see Table 2). Table 2 also gives the analytical figures of merit for each of the seven mixtures studied. A photodiode array detector (Model 990, Waters Chromatography, 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

Table 3

Accuracy and Precision of HPLC Methods Using Spiked Drug Samples

Mixture	Component (s)	Concn Added (μg/mL)	Concn. Found (μg/mL)[a]	Percent Error	RSD, %
1	Ondansetron	32.9	32.7 ± 0.19	0.61	0.58
		65.7	68.3 ± 0.45	3.96	0.66
	Neostigmine	4.21	4.07 ± 0.08	3.30	1.80
		8.43	8.30 ± 0.10	1.50	1.20
2	Ondansetron	20.09	20.05 ± 0.01	0.20	0.05
		53.58	57.49 ± 0.10	7.30	0.17
	Naloxone	1.98	2.09 ± 0.00	5.60	0.00
		5.27	5.36 ± 0.00	1.70	0.00
3	Ondansetron	7.52	7.85 ± 0.04	4.39	0.51
		15.04	15.04 ± 0.01	0.00	0.07
	Droperidol	9.33	9.38 ± 0.04	0.54	0.43
		18.65	18.73 ± 0.00	0.43	0.00
4	Ondansetron	0.40	0.42 ± 0.00	5.00	0.00
		0.99	0.98 ± 0.02	1.00	2.40
	Midazolan	1.00	1.00 ± 0.003	0.00	0.26
		2.51	2.47 ± 0.00	1.60	0.00
5	Ondansetron	6.75	7.13 ± 0.06	5.63	0.84
		13.50	14.13 ± 0.06	4.67	0.42
	Rantidine	83.3	87.7 ± 0.36	5.28	0.41
		166.6	176.5 ± 0.35	5.94	0.20
6	Ondansetron	3.36	3.26 ± 0.05	2.98	1.53
		13.43	13.96± 0.02	3.95	0.14
	Fentanyl	0.42	0.42 ± 0.01	0.00	1.48
		1.67	1.73 ± 0.01	3.60	0.58
7	Ondansetron	9.0	9.20 ± 0.03	2.20	0.33
		20.3	20.57 ± 0.59	1.30	2.87
	Alfentanyl	1.2	1.19 ± 0.01	0.80	0.84
		2.7	2.75 ± 0.01	1.85	0.36

[a] Mean ± S.D., based on n = 3.

wavelengths. These experiments were performed on solutions of each drug 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 HPLC methods were evaluated using spiked samples containing each analyte. The results for the seven mixtures studied are shown in Table 3. The data indicate that all of the procedures give acceptable accuracy and precision for the analytes in the mixtures studied.

Intraday and interday variabilities for each drug mixture expressed as % RSD are reported in Table 2. The intraday data was based on six (6) replicate injections within a given day (n=6) and the interday data was based on six (6) replicate injections of the drug mixture run on three different days (n=18).

In summary, HPLC methods have been developed to assay for ondansetron and selected medications in two component mixtures contained in 0.9% sodium chloride injection USP. The study reveals that these methods give good accuracy and precision and could be used to investigate the chemical stability of the analytes in each mixture.

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