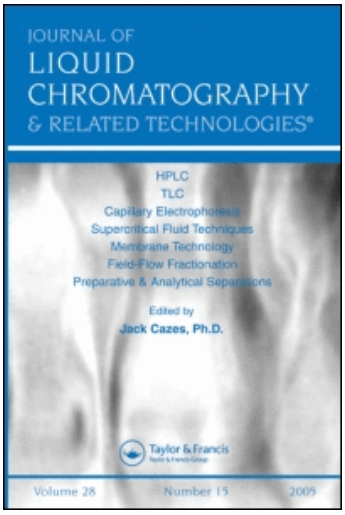

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HPLC Determination of Morphine-Hydromorphone-Bupivacaine and Morphine-Hydromorphone-Tetracaine Mixtures in 0.9% Sodium Chloride Injection

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HPLC DETERMINATION OF MORPHINE-HYDROMORPHONE-BUPIVACAINE AND MORPHINE-HYDROMORPHONE-TETRACAINE 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-hydromorphone-bupivacaine and morphine-hydromorphone-tetracaine mixtures in 0.9% sodium chloride injection. The separation and quantitation of morphine-hydromorphone-bupivacaine was performed on a phenyl column at ambient temperature using a mobile phase of 50:50 v/v 0.02M phosphate buffer pH 6.0-acetonitrile at a flow rate of 1.0 mL/min with the detection of all three analytes at 235 nm. The separation was achieved within 20 min with sensitivity in ng/mL range for each analyte. Morphine, hydromorphone and bupivacaine were linear in 5.0-51, 5.1-51.7 and 5.0-50 μg/mL ranges, respectively. Accuracy and precision were in the range 0.51-1.89 and 0.02-0.50%, respectively, for all three analytes and the limit of detection was close to 250 ng/mL for each component based on a signal to noise ratio of 3 and 20 μL injection. The separation and quantitation of morphine-hydromorphone-tetracaine was achieved on a silica column at ambient temperature using a mobile phase of 75:25 v/v 0.01 M phosphate buffer, pH 4.0-methanol at a flow rate of 1.0 mL/min with detection of the three analytes at 235 nm. The separation was achieved within 20 min with sensitivity in the ng/mL range for each analyte. Morphine, hydromorphone and tetracaine were

linear in the 4.95-49.6, 5.07-50.69 and 5.0-50.17 μg/mL ranges, respectively. Accuracy and precision were in the range 0.07-2.93% and 0.01-0.40%, respectively, for each analyte. The limit of detection was near 330 ng/mL for each compound and was based on a signal to noise ratio of 3 and a 20 μL injection.

INTRODUCTION

Morphine-hydromorphone-bupivacaine (A) and morphine-hydromorphone-tetracaine (B) mixtures are administered by epidural block for the treatment of pain. Interest in our laboratories in the stability and compatibility of the drug mixtures 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 all three compounds either in mixture A or mixture B concurrently in a single injection.

Hydromorphone, morphine and opiates, in general, have been analysed by HPLC with electrochemical (ECD) or UV detection (1-4) and by radioimmunoassay (RIA)(5,6). The HPLC methods are not as sensitive as RIA, but are more specific and often used in the analysis of the compounds. The HPLC-ECD method involved separation of hydromorphone and morphine on an octylsilane column equipped with an octadecylsilane guard column (1). The mobile phase consisted of 15:85 v/v absolute methanol-50 mM dibasic sodium phosphate pH 3.5 containing 3 mM octanesulphonic acid. The electrode potential was set at +600 mV vs Ag/AgCl. The HPLC-UV method for hydromorphone was achieved on an octadecylsilane column using a mobile phase of 40:1:0.5:58.5 v/v/v/v acetonitrile-anhydrous acetic acid-sodium dodecyl sulphate-water (3). The flow rate was 1.5 mL/min and the detector wavelength was 280 nm. Morphine has also been analysed using UV detection

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 (4). The wavelength for detection of the morphine was 210 nm. The RIA method utilized a commercially available RIA kit and detected hydromorphone in the 10-40 ng/mL range with a 0.1 mL serum sample (5,6).

Assay methods for bupivacaine include HPLC (7,8) GC (9,10) and amperometry (11). HPLC and GC are the most commonly reported methods for bupivacaine. The HPLC separation was achieved on an octylsilane column using a 92:8 v/v 10mM monobasic phosphate buffer pH 2.4-tetrahydrofuran mobile phase with detection at 210 nm. The flow rate was set at 1.6 mL/min. The GC procedure used nitrogen sensitive detection and was utilized in pharmacokinetic studies of bupivacaine.

Tetracaine has been analysed by derivative UV spectrophotometry (12) and HPLC (13,14). The spectrophotometric method was used to determine a cocaine and tetracaine mixture. A reversed phase HPLC method used an octadecylsilane column with a 70:30 v/v methanol-phosphate buffer pH 7.2 mobile phase and UV detection (13). An ion-pair chromatographic procedure used a mobile phase composed of acetonitrile, phosphate buffer, sodium chloride and tetrabutylamminium hydrogen sulphate (14). An octadecylsilane column was used for the separation and the tetracaine was detected at 294 nm.

In this paper, isocratic HPLC assays are presented for the simultaneous analysis of morphine, hydromorphone and bupivacaine (Mixture A) and

morphine, hydromorphone and tetracaine (Mixture B) in 0.9% sodium chloride injection. Mixture A was separated on a phenyl column using a buffered aqueous-acetonitrile eluent while Mixture B was separated on an underivatised silica column using a buffered aqueous-methanol eluent. Both separations were achieved within 20 min with sensitivities in the ng/mL range.

EXPERIMENTAL

Reagents and Chemicals

The structural formulae of the compounds studied are shown in Figure 1. Tetracaine and bupivacaine were purchased as their hydrochloride salts from Sigma Chemical Co. (St. Louis, MO 63178, Lot No. 102H0648 and 38F0507, respectively). Hydromorphone hydrochloride (Lot No. 31000144) was purchased from Knoll Pharmaceutical Company (Whippany, NJ 02101). Morphine sulfate reference standard was purchased from the United States Pharmacopeia (Rockville, MD 20852). Acetonitrile and methanol (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, potassium hydroxide and concentrated phosphoric acid were Baker analysed reagents.

Instrumentation

The chromatographic separation were performed on an HPLC system consisting of a Water 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). Separation of Mixture

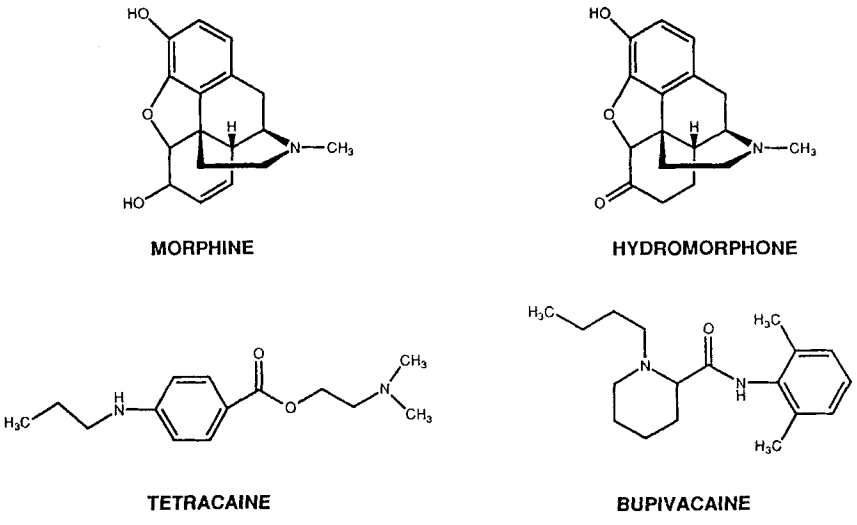

Figure 1 - Chemical structures of compounds studied.

A was achieved on a 30 cm phenyl column (3.9 mm i.d., 10 μm particle size, Waters μ Bondapak, Milford, MA 01757). The mobile phase consisted of 50:50 v/v 0.02M aqueous monobasic potassium phosphate, pH 6.0 (adjusted with 1N potassium hydroxide) -acetonitrile. The separation of Mixture B was accomplished on a 22 cm underivatized silica column (4.6 mm i.d., 5μm particle size, Brownlee Silica, Applied Biosystems, Inc., San Jose, CA 95134). The mobile phase consisted of 0.01M aqueous monobasic potassium phosphate pH 4.0 (adjusted with 10% phosphoric acid) - absolute methanol. The mobile phases were filtered through a 0.45 μm nylor-66 filter (MSI, Westborough, MA 01581) and degassed by sonication prior to use. The flow rate was 1.0 mL/min and the detector was set at 235 nm for both mixtrues.

Preparation of Standard Solutions

Combined standard solutions containing morphine, hydromorphone and tetracaine and morphine, hydromorphone and bupicacaine were prepared by accurately weighing 5 mg each of morphine sulfate, hydromorphone hydrochloride and tetracaine hydrochloride in a 100 mL volumetric flask and 5 mg each of morphine sulfate, hydromorphone hydrochloride and bupivacaine hydrochloride in a separate 100 mL volumetric flask. Fifty mL of 0.9% sodium chloride injection was added to each mixture and the flasks were shaken vigorously for 2 minutes folowed by addition of 0.9% sodium chloride to volume. These standard solutions, along with 1:10, 4:10 and 6:10 dilutions, gave solutions containing 5, 20, 30, and 50 μg/mL of each of the drugs as their respective salts. Four point calibration curves were constructed for each analyte using the analytical conditions established for each mixture. Additional dilutions (2:10 and 8:10) of each of the two combined 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

The goal of this study was to develop isocratic HPLC assays for the analytes of Mixture A and Mixture B in 0.9% sodium chloride injection within a 20 min time interval. Stability studies of these mixtures would require that assay procedures quantitate each analyte with reasonable accuracy and precision.

There were no reports in the scientific literature describing the separation of either combination of drugs in a single mixture. Initial studies to separate both analyte mixtures involved the use of an octadecylsilane column with various mobile phases containing acetonitrile-aqueous phosphate buffer. It was observed that there was great difficulty in separating the three components of either Mixture A or B within the desirable time interval. In addition, morphine and hydromorphone eluted close to the solvent front. When an ion pairing agent was added to the mobile phase, morphine and hydromorphone were retained longer and thus separated from the solvent front, but tetracaine or bupivacaine eluted late (> 30 min). Octylsilane columns were also investigated and essentially gave the same data as the octadecylsilane columns. With both octadecylsilane and octylsilane columns, tetracaine and bupivacaine eluted as broad peaks with extensive tailing. A deactivated octylsilane column provided sharp peaks for all the analytes but separation of either mixture was not accomplished within 20 min. Our attention turned towards a phenyl column. One commercial phenyl column gave good separation of the components, but at higher analyte concentrations gave rise to split peaks. However, another brand of phenyl column provided a very good separation of the components in Mixtures A and B. The best separations were obtained with a 50:50 v/v 0.02M aqueous phosphate buffer pH 6.0-acetonitrile. The run times for Mixture A and Mixture B were 20 and 25 min, respectively. An underivatized silica column was investigated to see if even faster run times could be obtained. Good separation of both Mixtures A and B were obtained using an aqueous phosphate buffer pH 4.0 with either acetonitrile or absolute methanol as organic modifier.

It was observed that there was better resolution and a faster turn-around time (< 20 min) when methanol was used as the organic modifier. Good separations were otobtained for both mixtures using a 75:25 v/v aqueous monobasic phosphate buffer pH 4.0-absolute methanol mobile phase. The run time was 25 min for Mixture A and 20 min for Mixture B. Since we were looking for run times of 20 min or less, we decided to use the phenyl column with a mobile phase of 50:50 v/v 0.02M phosphate buffer pH 6.0-acetonitrile to assay Mixture A and a silica column with a mobile phase of 75:25 v/v 0.01M phosphate buffer-methanol for the analysis of Mixture B. Typical chromatograms showing the separation of the analytes in Mixtures A and B under their respective analytical conditions are shown in Figures 2 and 3.

From earlier studies in this lab, it was shown that morphine and hydromorphone gave maximum UV absorbance at 235 nm in a acetonitrile phosphate buffer solvent system. Studies indicated that 210, 230 and 280 nm could also be used to detect bupivacaine and 235 and 294 nm for tetracaine. In acetonitrile - phosphate buffer mobile phases, the UV absorption maxima for tetracaine were 235 nm and 315 nm. Since there was good absorption of all analytes at 235 nm, the wavelength for detection of the analytes in both imxtures was set at 235 nm.

The HPLC method for Mixture A showed concentration versus absorbance linearity for morphine, hydromorphone and bupivacaine in the 5.0-51, 5.1-51.7 and 5.0-50 μg/mL ranges, respectively, at 235 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

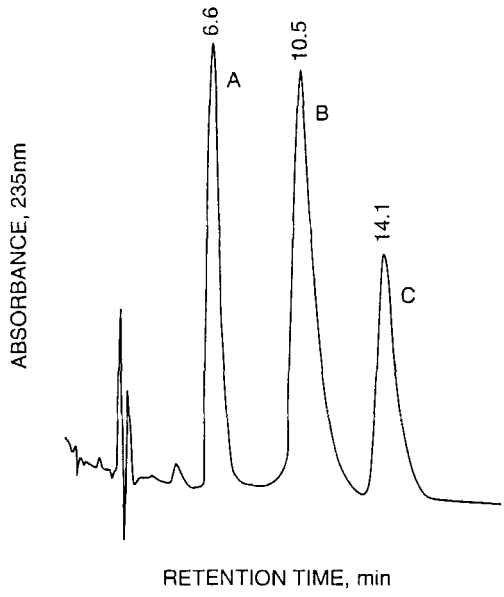

Figure 2 - Typical HPLC chromatogram of morphine (A), hydromorphone (B) and bupivacaine (C) on a phenyl column with 50:50 v/v 0.02 M phosphate buffer pH 6.0-acetonitrile. See Experimental Section for assay conditions.

morphine, hydromorphone and tetracaine in the 4.95-49.6, 5.07-50.69 and 5.0-50.17 μg/mL ranges, respectively, at 235 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 verify 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 235 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.

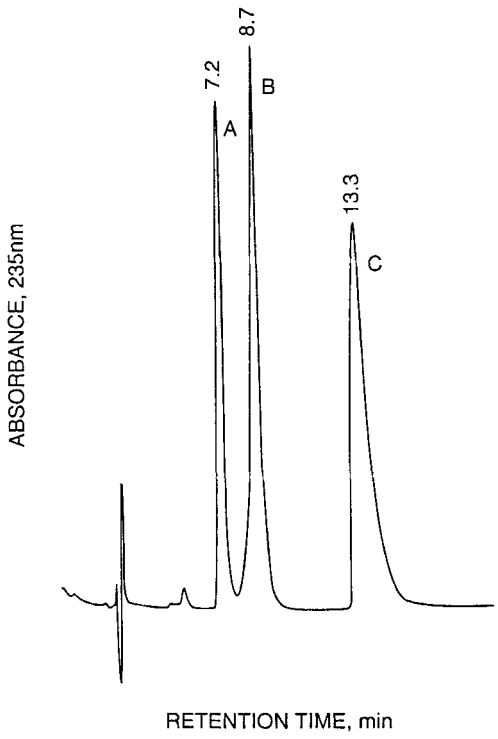

Figure 3 - Typical HPLC chromatogram of morphine (A), hydromorphone (B) and tetracaine (C) on a silica column with 75:25 v/v 0.01 M phosphate buffer pH 4.0-methanol. See experimental Section for assay conditons.

Percent error and precision of the methods were evaluated using spiked samples containing each analyte. The results for Mixture A and Mixture 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, hydromorphone and bupivacaine (Mixture A) expressed as % RSD were 0.6, 0.95 and 0.96% (n = 6), respectively. Inter-day variabilities of the assay for these drugs were in the 0.6-

Table 1

Analytical Figures of Merit for Morphine-Hydromorphone-Bupivacaine and Morphine-Hydromorphone-Tetracaine

Mixture	r^{2a}	System Suitability[b]	LOD ng/mL[c]	k'	Theoretical Plates[d]	Tailing Factor[e]	Rs
A							
Morphine	0.9975	0.60	258	1.34	1480	1.5	
							3.01
Hydromorphone	0.9993	0.95	253	2.31	1069	1.7	
							5.69
Bupivacaine	0.9998	0.96	254	4.97	2050	1.3	
B							
Morphine	0.9979	1.85	330	2.02	3006	1.2	
							2.63
Hydromorphone	0.9991	1.03	338	2.74	2053	1.1	
							4.87
Tetracaine	0.9996	1.49	334	5.15	1386	1.7	

[a] Range examined from 5.1-51 μg/mL morphine (n=9), 5.17-51.7 μg/mL hydromorphone (n=9) and 5.0-50.2 μg/mL bupivacaine for Mixture A at 235 nm and 4.9-49 μg/mL morphine (n=9), 5.0-50.6 μg/mL hydromorphone (n=9) and 5.0-50.1 μg/mL tetracaine (n=9) for Mixture B at 235 nm.

[b] Mean RSD % of 6 replicate injections at 51.0 μg/mL morphone, 51.7 μg/mL hydromorphone and 50.3 μg/mL bupivacaine for Mixture A at 235 nm and 49.5 μg/mL morphine, 50.7 μg/mL hydromorphone and 50.2 μg/mL tetracaine for Mixture B at 235 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

Mixture	Concn Added, μg/mL	Concn Found, μg/mL[a]	Percent Error	RSD (%)
A				
Morphine	10.21	10.30 ± 0.004	0.88	0.04
	40.84	41.05 ± 0.135	0.51	0.33
Hydromor-	10.35	10.46 ± 0.052	1.06	0.50
phone	41.40	41.81 ± 0.083	0.99	0.20
Bupivacaine	10.06	10.25 ± 0.016	1.89	0.16
	40.22	40.65 ± 0.006	1.07	0.015
B				
Morphine	9.89	9.82 ± 0.001	0.71	0.01
	39.56	40.72 ± 0.165	2.93	0.41
Hydromor-	10.14	10.22 ± 0.009	0.79	0.09
phone	40.55	40.87 ± 0.070	0.79	0.17
Tetracaine	10.03	9.96 ± 0.012	0.70	0.12
	40.14	40.17 ± 0.006	0.07	0.015

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

3, 0.95-1.6, 0.96-1.5 (n = 18 over 3 days) ranges, respectively. Intra-day variabilities for morphine, hydromorphone and tetracaine (Mixture B) expressed as % RSD were 1.58, 1.38 and 1.72% (n = 6), respectively. Inter-day variabilities of the assay for the analytes were in the 1.58-2.8, 1.0-2.7 and 1.49-2.55% (n = 12 over 3 days) ranges, respectively.

In summary, a phenyl column with an aqueous 0.02M pH 6.0 buffer-acetonitrile mobile phase was shown to be suitable for the separation and quantitation of a morphine-hydromorphone-bupivacaine mixture (A) in 0.9% sodium chloride injection. It has also been shown that a silica column with an

aqueous 0.01M phosphate buffer pH 4.0-methanol mobile phase is amenable for the separation and quantitation of a morphine-hydromorphone-tetracaine mixture (B). This study suggests that the above listed HPLC methods can be used to investigate the chemical stability of the three analytes in either Mixture A or B.

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