Simultaneous Quantitation of Morphine and Paraben Preservatives in Morphine Injectables

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Abstract □ A high-performance liquid chromatographic method for the simultaneous determination of morphine sulfate, methylparaben, and propylparaben in morphine sulfate injection was developed. A reversed-phase system, based on an octadecylsilane stationary phase, was used with a binary solvent mobile phase consisting of methanol–phosphate buffer (pH 4.0) containing methanol (5%) delivered at a constant rate (0.6:0.4 ml/min) using a two-pump system. The detector response at 254 nm was linear with the amount injected over a wide range, allowing rapid and reproducible quantitation of each component.

Keyphrases □ Morphine sulfate—high-performance liquid chromatographic analysis simultaneously with parabens in dosage form □ Methylparaben—high-performance liquid chromatographic analysis simultaneously with morphine sulfate and propylparaben in dosage form □ Propylparaben—high-performance liquid chromatographic analysis simultaneously with morphine sulfate and methylparaben in dosage form □ High-performance liquid chromatography—simultaneous analyses, morphine sulfate and parabens in dosage form □ Narcotic analgesics—morphine sulfate, high-performance liquid chromatographic analysis simultaneously with parabens in dosage form

Although neither the USP (1) nor the BP (2) requires the determination of preservative agents in official preparations of morphine sulfate injection, a simple method for the simultaneous quantitation of morphine and preservative agents occasionally is required. Official methods for the determination of morphine in pharmaceutical preparations are tedious and time consuming (3). Although column chromatographic–UV spectrophotometric methods were reported for the determination of paraben preservatives (4) and morphine in the presence of paraben preservatives (5), they also are time consuming. During a project to assess the effects of sterilization procedures on drugs used in anesthetic practice, an extremely simple and fast method was developed to quantitate simultaneously morphine, methylparaben, and propylparaben in morphine injectables using high-performance liquid chromatography (HPLC).

EXPERIMENTAL

Reagents and Chemicals—Analytical reagent grade chemicals were used. Methanol and aqueous solutions were filtered through a 0.45-μm filter[1] and degassed using an ultrasonic bath immediately before use.

Morphine sulfate[2], methylparaben[2], and propylparaben[2], all BP grade, were prepared for calibration by dissolving in distilled water, both singly and in admixture, in concentrations similar to the final concentration in the injectable—*viz.*, morphine sulfate, 10.0 mg/ml; methylparaben sodium, 0.63 mg/ml; and propylparaben sodium, 0.33 mg/ml.

Apparatus—A high-performance liquid chromatograph[3] was equipped with an octadecylsilane column[4] for reversed-phase chromatography and a recording digital integrator[5]. The chromatograph detector was set at 254 nm. The absorbance scale was set at 1.0 unit full scale, and the recorder was set at 1 cm/min.

Mobile Phase—The mobile phase was delivered from two pumps. Pump A delivered pure methanol. Pump B delivered an aqueous solution of 0.1% monobasic sodium phosphate dihydrate containing 5% methanol at pH 4.0 (the pH was adjusted by dropwise addition of either 1% phosphoric acid or 1% dibasic sodium phosphate). A total flow rate of 1.0 ml/min consisted of 0.6 ml/min from Pump A and 0.4 ml/min from Pump B.

Determination of Calibration Curve—Triplicate volumes of each standard solution (1.0–10.0 μl), first individually and then in admixture, were injected into the chromatograph using a 10-μl microsyringe. Regression of the detector response for each component on volume injected was assessed in two ways: by manually measured peak heights and by electronically computed peak areas.

Determination of Components in Morphine Sulfate Injection—Exactly 3.0-μl aliquots of morphine sulfate injection (nominally containing 10.0 mg of morphine sulfate/ml, 0.63 mg of methylparaben so-

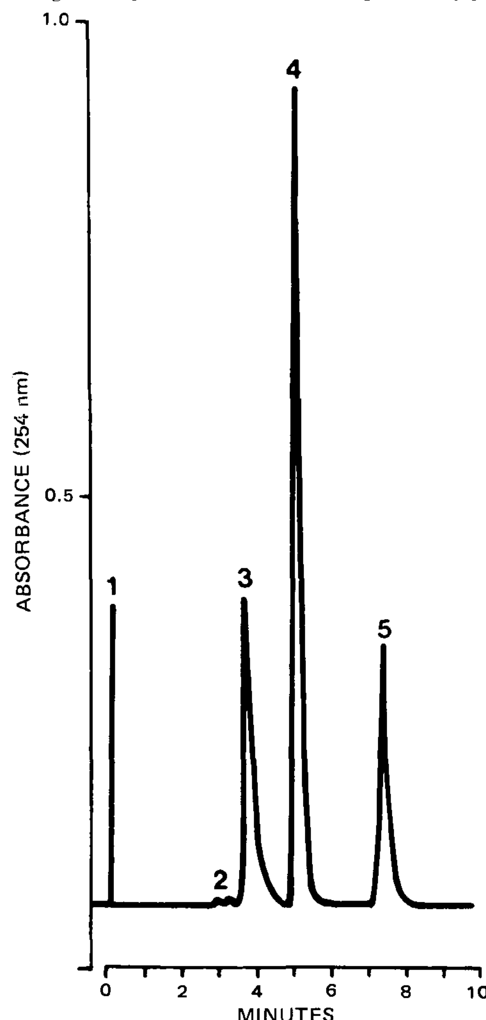

Figure 1—*High-pressure liquid chromatogram obtained with the described method. Key: peak 1, point of injection; peak 2, trace components found in morphine sulfate injections; peak 3, morphine; peak 4, methylparaben; and peak 5, propylparaben.*

[1] Gelman Metricel GA-6.
[2] Courtesy of David Bull Laboratories, Melbourne, Australia.
[3] Waters ALC/GPC 200 series with U6K universal injector, model 440 multiple wavelength absorbance detector, model M6000A pumps, and model 660 solvent programmer.
[4] μBondapack C_{18}, 10-μm particle size, 4 mm i.d. × 30 cm.
[5] Hewlett-Packard model 3380A.

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Table I—Regression Analysis of Detector Response (254 nm) Measured as Peak Height and Peak Area on Volume Injected

	Morphine	Methyl-paraben	Propyl-paraben
Peak height			
Intercept, mm	7.4	−2.5	−1.2
Slope, mm/μl	11.71	34.21	10.25
r^2	0.99406	0.99915	0.99731
Range, μl	1.0–10.0	1.0–7.0[a]	1.0–10.0
n	30	21	30
Peak area			
Intercept, (mv)(sec)	−1933	3840	−1386
Slope, (mv)(sec)/μl	20,769	31,285	15,214
r^2	0.99984	0.99581	0.99826
Range, μl	1.0–10.0	1.0–6.0[b]	1.0–10.0
n	30	18	30

[a] The response beyond 7.0 μl was off scale. [b] The response was nonlinear beyond 6.0 μl.

dium/ml, and 0.33 mg of propylparaben sodium/ml) were injected directly into the chromatograph. Peak area calibration curves were used to read off the equivalent volume injected (and, therefore, the drug amount injected). The results were calculated using:

$$\text{percent of strength claim} = \frac{V_0}{V_i} \times 100\% \qquad \text{(Eq. 1)}$$

where V_0 is the volume observed as equivalent from standard curve and V_i is the volume actually injected.

Effects of Sterilization on Active Ingredients—Ampuls from a test batch of morphine sulfate injection were obtained both before and after routine factory sterilization. Representative samples from each lot were divided into two groups and then subjected to three cycles of sterilization by autoclaving at 120–121° for 30 min. One group from each lot was cooled rapidly over 5–10 min while the other group was cooled slowly over 45–60 min.

Analysis of variance was used to detect any differences between the procedures.

RESULTS AND DISCUSSION

Mobile Phase—HPLC provides an excellent method of separation and quantitation of these compounds, which differ markedly in volatility and polarity. The analysis conditions finally chosen for routine use fulfilled the requirements of simplicity, speed, and separation. By varying the binary mixture, other drugs including lidocaine, mepivacaine, bupivacaine, and meperidine may readily be analyzed using the same combination of column and solvent systems. The separation of a wide range of chemical types using an octadecylsilane stationary phase with a methanol-water mobile phase has been reported (6).

Optimum operating conditions depend on the mobile phase pH. Operation at pH 4.0 produced symmetrical peaks for all components (Fig. 1). Preliminary studies using an unbuffered aqueous phase and aqueous phosphate buffer at pH 6.0 or 8.0 or an ammonium carbonate solution produced insufficiently symmetrical peaks for reproducible quantitation. Hays *et al.* (7) reported unsuccessful quantitation chromatography of morphine under a variety of mobile and stationary phase conditions because of peak asymmetry.

Liquid chromatography of weak bases such as morphine (pKa = 8.0) presents special problems because of the pH-dependent dissociation of the conjugate acid. This dissociation may be overcome using ion suppression techniques by operating at a pH where the free base is preponderant. For morphine, operation in excess in pH 10 would be required, but it is not feasible because stationary phase decomposition occurs at pH 8 or greater. Recently, ion-pair techniques were developed to enable optimum separation of symmetrical peak shapes. The separation presently described separates the hydrophilic conjugate acid of morphine from the more hydrophobic neutral paraben esters. Under these conditions, morphine is retained on the column (k' = 0.4) long enough for highly reproducible separation and quantitation.

Table II—Reproducibility of 10 Replicate Simultaneous Determinations of Morphine, Methylparaben, and Propylparaben

Variable	Mean	CV, %	Range
Morphine			
Retention time, min	3.57	1.2	3.53–3.64
Peak height, mm	53.70	1.4	53.0–54.7
Peak area, (mv)(sec)	71,924	0.7	71,287–73,156
Methylparaben			
Retention time, min	5.27	0.4	5.22–5.29
Peak height, mm	120.70	0.7	119.5–121.7
Peak area, (mv)(sec)	118,352	0.6	117,687–119,521
Propylparaben			
Retention time, min	8.25	1.3	8.11–8.37
Peak height, mm	33.80	1.2	33.2–34.5
Peak area, (mv)(sec)	51,320	0.6	50,894–51,799

Table III—Concentrations of Morphine and Paraben Preservatives in Ampuls after Three Cycles of Pharmacy Autoclave Sterilization

	Not Factory Sterilized, Mean ± SD, mg/ml	Factory Sterilized, Mean ± SD, mg/ml
Control		
Morphine sulfate	11.31 ± 0.23	11.30 ± 0.45
Methylparaben sodium	0.76 ± 0.03	0.76 ± 0.04
Propylparaben sodium	0.38 ± 0.01	0.38 ± 0.00
Rapid cool		
Morphine sulfate	11.18 ± 0.50	10.92 ± 0.32
Methylparaben sodium	0.77 ± 0.05	0.76 ± 0.04
Propylparaben sodium	0.37 ± 0.01	0.39 ± 0.00
Slow cool		
Morphine sulfate	10.84 ± 0.35	11.35 ± 0.34
Methylparaben sodium	0.76 ± 0.05	0.77 ± 0.06
Propylparaben sodium	0.37 ± 0.01	0.38 ± 0.01

While determining optimum separation conditions, a problem of spurious detector response was encountered consistently at fractional flows of methanol between 20 and 80%. Both random fast and systematic slow noise signals were observed. This effect was attributed to the liberation of gas on mixing of the solvents. The liberated gas affected the constant flow rate solvent delivery system and occurred even with freshly degassed methanol and aqueous solutions. The problem was circumvented by the addition of 5% methanol to the aqueous phase prior to degassing.

Calibration Curve and Reproducibility—There is a longstanding dispute over the choice between peak height and peak area measurements in chromatographic determinations. For two of the three components, marginally higher correlation coefficients were obtained from analysis of the regression of peak area on volume injected when compared to the corresponding analysis using peak heights (Table I). A definite discontinuity appeared in the peak height calibration curve for morphine, which was not reflected in the peak area curve. Although injection of volumes greater than 7.0 μl of methylparaben resulted in peaks being off scale, they were still integrated. The peak area–volume injected relationship was linear to 6–7 μl injected; beyond this limit, distinct nonlinearity was observed. Both the peak height and peak area *versus* volume injected standard curves were linear for propylparaben over the 0–10-μl range tested.

Details of a reproducibility study for 10 replicate determinations are given in Table II. The method appears quite robust in that retention times of the compounds were very consistent with coefficients of variation in the order of 1% for all three components.

Excellent quantitative reproducibility was obtained using both peak height and peak area measurements. Coefficients of variation for peak height measurements were approximately double those for computed peak areas, confirming the preference for the latter method of calculation.

Effect of Sterilization Procedure—There were no significant differences between the two rates of cooling with respect to active ingredient concentration. No degradation of active ingredients was observed for any component measured (Table III).

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