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Quantitative Determination of Morphine in Paregoric USP by High-Pressure Liquid Chromatography

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Abstract □ The quantitative determination of morphine in paregoric by reversed-phase high-pressure liquid chromatography is described. The method is simple, accurate, and fast compared to the USP method. Benzoic acid in paregoric also can be determined quantitatively with the same column. The method was applied to two commercial samples with excellent results.

Keyphrases □ Morphine—high-pressure liquid chromatographic analysis in commercial samples of paregoric □ High-pressure liquid chromatography—analysis, morphine in commercial samples of paregoric □ Paregoric—high-pressure liquid chromatographic analysis of morphine and benzoic acid, commercial samples □ Analgesic agents—morphine, high-pressure liquid chromatographic analysis in commercial samples of paregoric

The USP method (1) for the quantitative determination of morphine in paregoric is tedious and time consuming. No other easy method is available for determining morphine in paregoric USP. The purpose of these investigations was to develop a simple, accurate, and fast method for the analysis of morphine using high-pressure liquid chromatography (HPLC). A method for the quantitative determination of benzoic acid in paregoric using the same column also is described.

EXPERIMENTAL

Apparatus—A high-pressure liquid chromatograph[1] capable of operating at an inlet pressure up to 6000 psig was used.

Column—The column[2] (30 cm long and 4 mm i.d.) was purchased and used as received. It consisted of a monomolecular layer of octadecyltrichlorosilane permanently bonded to silica *via* silicon–carbon bonds.

Recorder—The recorder[3] was equipped with an integrator.

Chromatographic Conditions—The chromatographic solvents were: (*a*) 0.1 *M* KH_2PO_4 buffer solution in 7% (v/v) methanol in water for morphine and (*b*) 0.1 *M* KH_2PO_4 buffer solution in 10% (v/v) methanol in water for benzoic acid. The temperature was ambient. The flow rate was 1.8 ml/min (inlet pressure approximately 1000 psig) for morphine or 3 ml/min (inlet pressure approximately 2100 psig) for benzoic acid. The absorbance unit full scale was 0.16, and the chart speed was 30.5 cm (12 in.)/hr.

Chemicals and Reagents—All chemicals and reagents were USP, NF, or ACS grade. Morphine sulfate[4] USP and all other reagents were used without further purification.

Solutions—*Benzoic Acid Standard Solution*—Weigh 100.32 mg of benzoic acid, dissolve in 15.0 ml of approximately 0.1 *N* NaOH solution, and dilute to volume (100.0 ml) with water.

Morphine Standard Solution—Weigh 53.3 mg of morphine sulfate (equivalent to 40.0 mg of anhydrous morphine) and dissolve in enough water, containing 8.0 ml of approximately 0.1 *N* H_2SO_4, to bring to 250.0 ml.

Standard Mixture Similar to Paregoric—Dissolve 53.3 mg of morphine sulfate in 4–5 ml of water; dissolve 0.38 ml of anise oil, 0.38 g of benzoic acid, 0.38 g of camphor, and 3.8 ml of glycerin in 48 ml of alcohol. Mix the aqueous and alcoholic solutions and dilute to volume (100.0 ml) with water.

Assay Procedure for Morphine in Standard Mixture and Paregoric—Transfer 10.0 ml of the standard mixture or paregoric to a 150-ml beaker and add 2.0 ml of ~0.1 *N* H_2SO_4 and 20 ml of water. Boil on a hot plate until the volume is approximately 8 ml, cool, and dilute to volume (25.0 ml) with water. Filter if necessary and inject 40.0 μl, using the described chromatographic conditions.

The detector response to the standard mixture and one commercial sample is presented in Fig. 1. Since preliminary investigations indicated that concentration (4–8 μg) was directly related to the peak area,

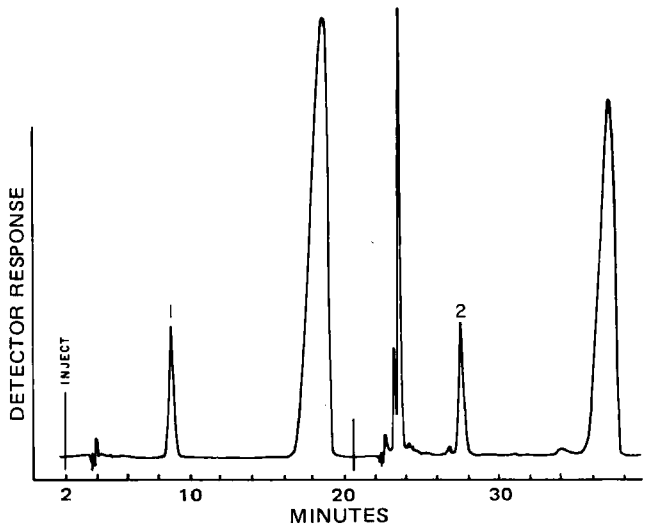

Figure 1—*HPLC analysis of morphine in paregoric USP (for chromatographic conditions, see text). Key: 1, standard mixture; and 2, paregoric sample.*

[1] Waters ALC 202 equipped with U6K Universal liquid chromatograph injector and UV detector (254 nm), Waters Associates, Milford, Mass.
[2] μBondapak/C_{18}, Waters Associates, Milford, Mass.
[3] Omniscribe model 5213-12, Houston Instruments, Austin, Tex.
[4] Merck & Co.

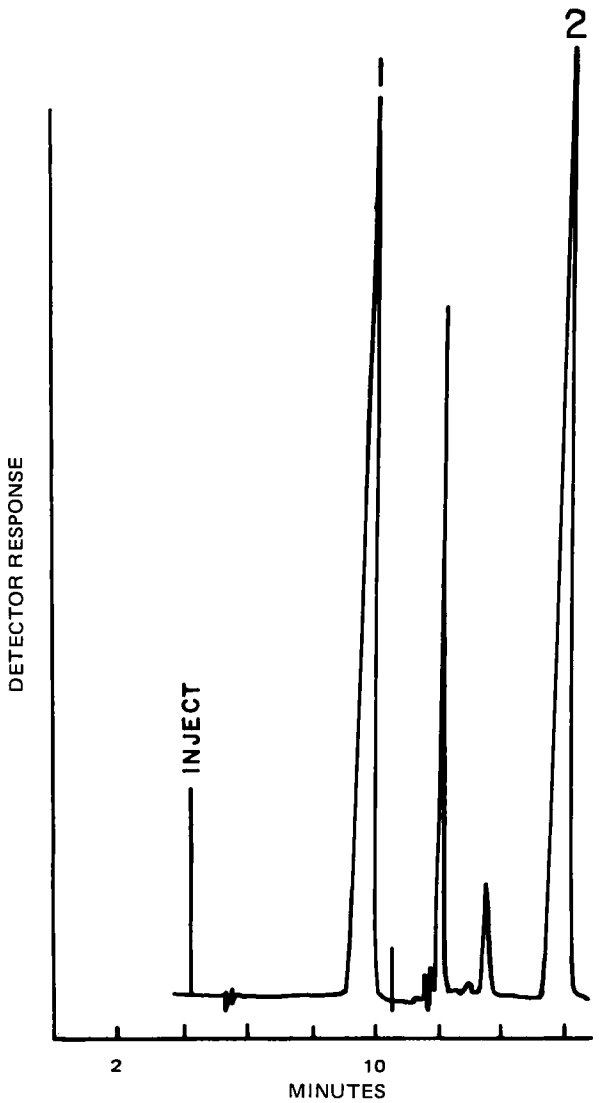

Figure 2—*HPLC analysis of benzoic acid in paregoric USP (for chromatographic conditions, see text). Key: 1, standard solution; and 2, commercial paregoric sample.*

the results on commercial samples were calculated by comparing the peak areas of morphine using:

$$\frac{A_a}{A_s} \times 100 = \text{percent of claim} \qquad \text{(Eq. 1)}$$

where A_a is the peak area of the assay, and A_s is the peak area of the standard. The time of retention and the peak for morphine from the morphine standard solution were identical to those from the standard mixture.

Table I—Assay Results on Two Commercial Samples of Paregoric

Sample	Morphine[a], %	Benzoic Acid[a], %
Standard mixture	100.4	99.7
I	104.3[b]	101.4
II[c]	105.5[d]	101.8
Average deviation	±2.6	±1.9
SD	3.22	2.35

[a] Average of three. [b] The manufacturer reported assay results of 105.3% using the USP (2) method. [c] From a different manufacturer. [d] The manufacturer reported assay results of 107.0% using the modified USP method.

Assay Procedure for Benzoic Acid—Transfer 6.6 ml of paregoric to a 150-ml beaker and add 20 ml of water and 3.1 ml of ~0.1 *N* NaOH. Boil on a hot plate until the volume is approximately 8 ml, cool, dilute to volume (25 ml) with water, and inject 20.0 μl.

The detector response to a commercial sample is presented in Fig. 2. Since preliminary investigations indicated that concentration (12–25 μg) of benzoic acid was directly related to peak area, the results were calculated by comparing the peak area of the unknown sample with that of the standard (Table I).

DISCUSSION

The assay results (Table I) on morphine and benzoic acid indicate that HPLC can be used for the quantitative analysis of these ingredients in commercial samples of paregoric. At least in a twofold range, the areas of peaks were related to the concentrations of both morphine and benzoic acid. This result was considered satisfactory for analysis. In the case of morphine, the time required was about 20 min *versus* about 4–6 hr by the USP method (1). The ratio of methanol and the flow rate (1.8 ml/min) were adjusted to separate the small peak immediately on the left side of the morphine peak (Fig. 1). This peak must be separated from the morphine peak or the results may be high. This was a major problem, especially with Sample II.

As far as benzoic acid is concerned, the conditions (*e.g.*, lesser flow rate or higher percent of methanol) may be changed without difficulties in separation.

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