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Determination of Ergometrine Maleate and Methylergometrine Maleate in Pharmaceutical Preparations by High-Performance Liquid Chromatography

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A high-performance liquid chromatographic method for the determination of ergometrine maleate (EM) and methylergometrine maleate (MM) in pharmaceutical preparations was established. EM and MM were extracted with dichloromethane from injections and tablets in the presence of sodium chloride and ammonia solution. After evaporation of the dichloromethane, the residue was dissolved in water. Then EM and MM were determined by high-performance liquid chromatography on a LiChrosorb RP-18 column using a mixture of acetonitrile and 50 mM acetate buffer (pH 3.5) containing 1.5 mM triethylamine. In comparing the analytical data obtained by this method with those by the colorimetric method, both data were in excellent agreement except in the case of MM tablets, which contained related compounds. EM in the solution prepared according to the content uniformity test for EM tablets in JP X was determined by both methods. The average values of the contents in 10 tablets were 96.0% by the colorimetric method and 80.8% by the HPLC method. The degradation of EM took place during shaking for the extraction of EM and could not be detected by the colorimetric method.

Keywords——ergometrine maleate; methylergometrine maleate; high-performance liquid chromatography; colorimetry

Ergometrine maleate (EM) and methylergometrine maleate (MM) in pharmaceutical preparations used as uterine stimulants are determined by a colorimetric method with *p*-dimethylaminobenzaldehyde (*p*-DBA) in many pharmacopeias.[1] Since related compounds and degradation products of EM and MM also react with *p*-DBA,[2] a more specific assay method is desirable.

Several authors[3] have reported high-performance liquid chromatographic (HPLC) studies on ergot alkaloids. For instance, Sondack[3b] and Pask-Hughes *et al.*[3d] directly injected the aqueous solution extracted from EM tablets and injections (containing oxytocin) into an HPLC column. However, their methods suffer from interference by excipients and stabilizing agents present in pharmaceutical preparations.

This paper describes a new HPLC assay method for EM and MM in various pharmaceutical preparations on a LiChrosorb RP-18 column using a mixture of acetonitrile and 50 mM acetate buffer (pH 3.5) containing 1.5 mM triethylamine after extraction with dichloromethane.

Analytical data obtained by this method were compared with those by the colorimetric method,[1a] and good agreement was obtained, except for one sample. The content uniformity test for EM tablets was newly adopted in JP X.[1a] An attempt to apply this HPLC method to the content uniformity test was made. When each tablet was shaken in the tartaric acid solution of the JP X content uniformity test for EM tablets, the degradation of EM occurred during the shaking. This degradation could not be detected by the colorimetric method in JP X.

Experimental

Apparatus and Chromatographic Conditions——The liquid chromatograph consisted of an Atto ultraviolet (UV) detector (254 nm), a Hitachi 650-10S fluorescence spectrophotometer (excitation wavelength of 314 nm and emission wavelength of 424 nm) with a Nihon Seimitsu NSP 800-9 high-pressure pump, and a Shimadzu C-R1B Chromatopac. A stainless steel column (150 × 4 mm i.d.) was packed with LiChrosorb RP-18 (particle size, 5 μm; Merck Co.) and the column temperature was maintained at 30 °C. The pressure was 100 kg/cm^2, giving a flow rate of 1 ml/min. Mixtures of acetonitrile and 50 mM acetate buffer (pH 3.5) containing 1.5 mM triethylamine were used as a mobile phase for EM and MM in mixing ratios of 45 : 55 and 40 : 60 (v/v), respectively.

Absorbance was measured with a Hitachi 139 spectrophotometer using a quartz cell of 1 × 1 cm optical pathlength.

Reagents and Solutions——The EM reference standard adopted was the Japanese Pharmacopeia Reference Standard, and EM was the products of Sigma Co. and Sandoz Co. MM was a gift from Mochida Pharmaceutical Co. EM and MM tablets and injections were purchased locally. 17α-Hydroxyprogesterone was the product of Sigma Co. All other reagents were of reagent grade.

Ammonia Solution: 40 ml of strong ammonia water was diluted to 100 ml with water (10%).

Internal standard (IS) Solution: 10 mg of 17α-hydroxyprogesterone was dissolved in methanol to give a concentration of 20 or 30 μg/ml.

Standard Solution of EM or MM: About 10 mg of EM reference standard or bulk MM, previously dried over silica gel for 4 h and accurately weighed, was dissolved in water and made up to 250 ml (0.04 mg/ml).

Assay by the HPLC Method——EM: About 10 mg of EM, previously dried over silica gel for 4 h and accurately weighed, was placed in a 250 ml volumetric flask and water was added to volume. Then 2.0 ml of this solution was mixed with 2.0 ml of IS solution at 20 μg/ml, and a 20 μl aliquot was injected into the HPLC column.

EM and MM Injection: A mixture of 1.0 ml of injection (0.2 mg/ml), 0.3 g of sodium chloride and 0.2 ml of ammonia solution was shaken vigorously for 10 min with 5.0 ml of dichloromethane. After standing for a few min, 4.0 ml of the dichloromethane layer was evaporated to dryness under a current of nitrogen, and the residue was dissolved in 4.0 ml of water. Next, 2.0 ml of this solution was mixed with 2.0 ml of IS solution at a concentration of 20 μg/ml in the case of EM and 30 μg/ml in the case of MM. A 20 μl aliquot was injected into the HPLC column. The peak heights were measured and the ratios of the peak heights of EM and MM were calculated with respect to that of IS.

$$\text{amount of EM or MM (mg)} = \text{amount of EM reference standard or bulk MM (mg)} \times H_T/H_S \times 1/50$$

H_T and H_S are the ratios of the peak heights obtained from the sample and standard solution of EM or MM.

EM and MM Tablets: Not less than 20 tablets were accurately weighed and powdered. A portion of the powder containing about 0.2 mg of EM or MM was accurately weighed and placed in a glass-stoppered test tube. After adding 1 ml of water, the tube was ultrasonicated for 2 min, then 0.3 g of sodium chloride, 0.2 ml of ammonia solution and 5.0 ml of dichloromethane were added. The following procedure was the same as in the case of EM and MM injection.

Colorimetric Method——The assay procedures for EM, MM, their tablets and EM injection are described in JP X,[1a] and that for MM injection in USP XX.[1b]

Results and Discussion

Conditions of the HPLC Method

The commercial EM injection contained several other ingredients, such as ascorbic acid, benzyl alcohol, *etc.* Therefore, mixtures of EM, MM, IS, benzyl alcohol and ascorbic acid in concentrations of 20, 20, 15, 125 and 125 μg/ml, respectively, were prepared. As shown in Fig. 1, ascorbic acid was always eluted at the position of the dead volume; the retention times (t_R) of EM, MM, IS and benzyl alcohol were examined.

Column Temperature——The effect of the column temperature on t_R was examined in the range of 20 to 45 °C. An increase of the column temperature slightly shortened the t_R of each component. Thus the column temperature was set at 30 °C.

Concentration of Triethylamine——Figure 2 shows that the t_R values of IS and benzyl alcohol were constant, but that those of EM and MM changed markedly. To investigate the effect of amine or ammonium group, 1.5 mM tetramethyl ammonium hydroxide, monoethylamine and ammonium sulfate were used. With triethylamine, tetramethyl ammonium hy-

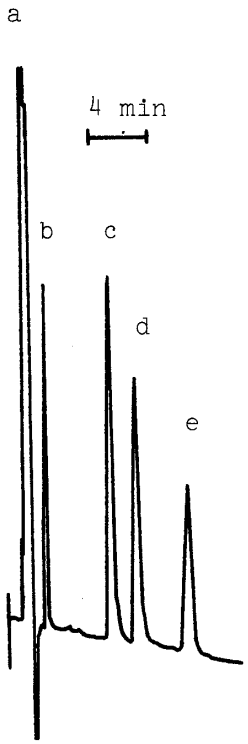

Fig. 1. High-Performance Liquid Chromatogram for EM, MM, Internal Standard, Ascorbic Acid and Benzyl Alcohol

Conditions: column, LiChrosorb RP-18 (5 μm), 150 × 4 mm i.d.; mobile phase, a mixture of acetonitrile and 50 mM acetate buffer (pH 3.5) (40 : 60) containing 1.5 mM triethylamine; flow rate, 1 ml/min; detection, 254 nm; column temperature, 30 °C.
a, ascorbic acid; b, benzyl alcohol; c, EM; d, MM; e, internal standard.

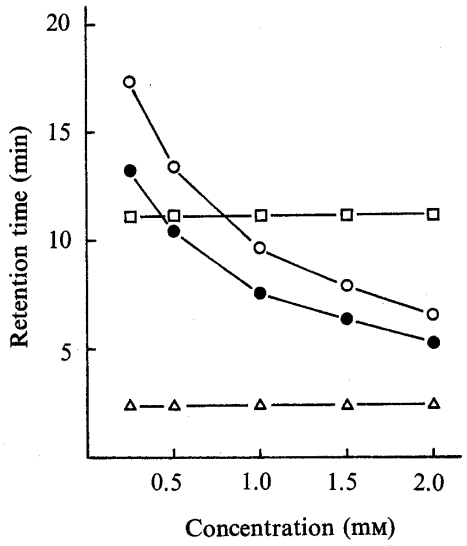

Fig. 2. Effect of Triethylamine

●, EM; ○, MM; △, benzyl alcohol; □, internal standard.

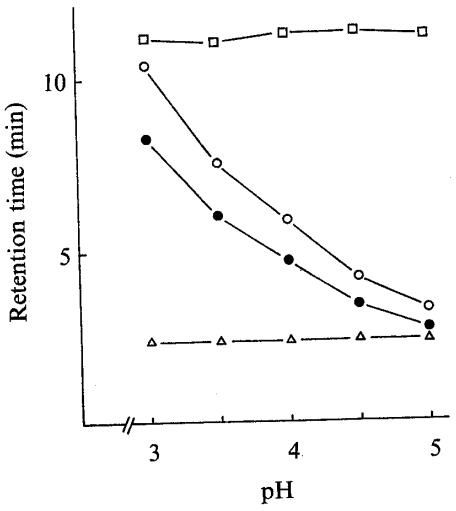

Fig. 3. Effect of pH

●, EM; ○, MM; △, benzyl alcohol; □, internal standard.

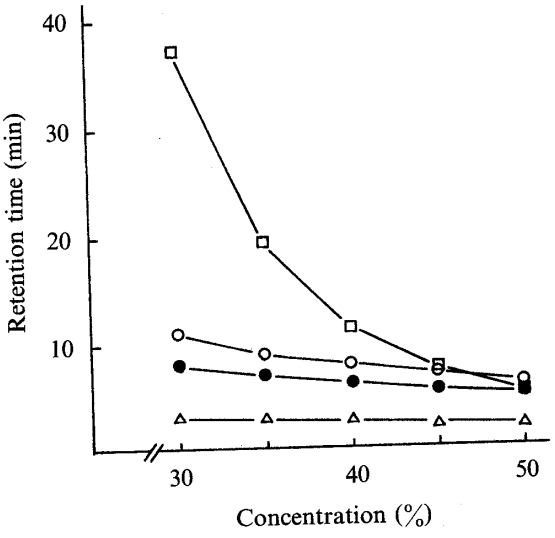

Fig. 4. Effect of Acetonitrile

●, EM; ○, MM; △, benzyl alcohol; □, internal standard.

droxide, monoethylamine and ammonium sulfate, the t_R values of EM were 6.4, 6.9, 13.6 and 9.5 min, respectively, and those of MM were 8.0, 8.8, 18.9 and 12.8 min, respectively. Triethylamine had the greatest effect on the t_R values of EM and MM; 1.5 mM triethylamine

was considered to be suitable.

Ionic Strength——The effect of ionic strength of acetate buffer was examined in the concentration range of 25 to 100 mM. With increasing ionic strength of buffer, the t_R values of IS and benzyl alcohol were constant, but those of EM and MM were shortened (7.0 and 8.7 min at 25 mM, respectively, and 3.9 and 4.8 min at 100 mM, respectively); 50 mM acetate buffer was selected.

pH——Figure 3 showed that the t_R values of EM and MM were affected significantly. The pH was set at 3.5.

Concentration of Acetonitrile——As shown in Fig. 4, an increase of the concentration of acetonitrile caused a large shortening of the t_R of IS in comparison with the t_R values of benzyl alcohol, EM and MM. An acetonitrile concentration of 45% was selected in the case of EM and 40% in the case of MM.

Working Curve and Reproducibility——The working curves for EM and MM were linear from 0 to 30 μg/ml and passed through the zero point. Their coefficients of correlation were 0.9999 and 0.9998, respectively.

At concentrations of 5 and 20 μg/ml of EM, the average values of the ratios of the peak height and their coefficients of variation were 29.0±3.8% ($n=6$) and 122.9±1.1% ($n=6$), respectively. At 5 and 20 μg/ml of MM, the values were 19.7±2.3% ($n=6$) and 80.4±2.1%, respectively.

Application to Pharmaceutical Preparations

EM and MM injection (0.2 mg/ml) contained 0.15 M sodium chloride (0.9%) to make the solution isotonic. Mixtures of EM at 20 μg/ml and IS at 10 μg/ml in the presence of 0 to 50 mM sodium chloride were prepared and chromatographed. The ratios of the peak height of EM in the presence of 5, 10, 20 and 50 mM sodium chloride to those of EM in the absence of sodium chloride were 101.0, 102.7, 103.6, 103.7 and 109.6%, respectively. Thus EM and MM had to be extracted from the aqueous solution with organic solvent instead of being directly applied. In the assay of EM tablets in J.P. X,[1a] EM was extracted from the aqueous solution with diethyl ether. To apply this extracting procedure in our method, we further investigated the extracting conditions. When 5 ml of diethyl ether or dichloromethane was used, the recoveries of EM were 86.3 and 99.2%, respectively. Thus, 5 ml of dichloromethane was selected.

Sodium Chloride——At 0, 0.1, 0.2, 0.3 and 0.4 g of sodium chloride, the recoveries of EM were 89.4, 99.8, 101.1, 100.4 and 103.5%, respectively, and those of MM were 81.0, 93.5, 101.0, 100.6 and 94.8%, respectively. With 0.2 and 0.3 g of sodium chloride, EM and MM were completely extracted. Thus, 0.3 g of sodium chloride was selected.

Ammonia Solution——At 0, 0.1, 0.2 and 0.3 ml of ammonia solution, the recoveries of EM were 19.7, 100.4, 99.2 and 100.6%, respectively, and those of MM were 40.4, 101.0, 99.9 and 98.6%, respectively. The use of 0.1 to 0.3 ml of ammonia solution gave complete extraction of EM and MM; thus, 0.2 ml of ammonia solution was chosen.

Shaking Time——At shaking times of 5, 10 and 15 min, the recoveries of EM were 99.1, 100.4 and 99.4%, respectively, and those of MM were 99.5, 100.5 and 100.2%, respectively. A shaking time of 10 min was selected.

Recovery——The recoveries of EM and MM were examined at concentrations of 0.05 and 0.2 mg/ml. EM and MM were recovered quantitatively from the aqueous solution, and the coefficients of variation were less than 2.9% ($n=6$).

Determination of EM and MM in Pharmaceutical Preparations by the HPLC Method and the Colorimetric Method——As shown in Table I and II, the analytical data obtained by both methods showed excellent agreement except in the case of MM tablet (G).

When MM tablets were checked by the HPLC method, three peaks appeared on the chromatogram. The first (t_R 8.2 min) and the second peak (t_R 10.8 min) coincided with those of

Table I. Content of EM in Pharmaceutical Preparations Determined by the HPLC Method and Colorimetric Method

		Content of EM (%)	
		HPLC method	Colorimetric method
Bulk	A	100.7	100.2
	B	101.3	100.2
Tablet	C[a]	94.0	95.6
Injection	D[b]	86.7	83.8
	E[b]	101.8	100.8

a) One tablet contains 0.5 mg of EM.
b) 1.0 ml of injection contains 0.2 mg of EM, 5 mg of ascorbic acid, 5 mg of sodium chloride and 10 mg of benzyl alcohol.

Table II. Content of MM in Pharmaceutical Preparations Determined by the HPLC Method and Colorimetric Method

		Content of MM (%)	
		HPLC method	Colorimetric method
Bulk	F	—	100.1
Tablet	G[a]	88.6	96.3
Injection	H[d]	100.4	99.6
	I[d]	100.9	100.8

a) One tablet contains 0.125 mg of MM.
b) 1.0 ml of injection contains 0.2 mg of MM and 5 mg of sodium chloride.

MM and IS. The third peak (t_R 13.6 min) was thought to be that of a related compound. The ergot alkaloids show fluorescence. Thus, to check whether the third peak was a kind of alkaloids, the eluate was monitored with a fluorospectrophotometer. Two fluorescent peaks appeared at the positions of the first and third peaks; both peaks had a maximum excitation wavelength of 314 nm and a maximum emission wavelength of 424 nm. This suggested that the compound of the third peak had an ergot alkaloid structure. Therefore, the analytical values obtained by the colorimetric method should be higher than those by the HPLC method.

The content uniformity test for EM tablets was adopted in JP X.[1a)] EM is determined colorimetrically after extracting EM in one tablet with 1% tartaric acid solution for 30 min. The contents of EM in each of 10 tablets were assayed by the colorimetric method and the HPLC method using the solution prepared under the procedure of the content uniformity test in JP X. The average values of the contents were 96.0% by the colorimetric method and 80.0% by the HPLC method, and their coefficients of variation were 0.9 and 6.9%, respectively. To investigate the difference in the values, the effect of the shaking time in 1% tartaric acid solution was examined. At 10, 20, 30, 40, 50 and 60 min, the content of EM in 1% tartaric acid solution extracted from the powder of tablets gave a constant value of 96% by the colorimetric method, but the values obtained by the HPLC method were 90.8, 86.6, 83.6, 79.0, 75.3 and 71.3%, respectively. When EM in 1% tartaric acid solution was determined by the HPLC method, EM was stable for at least 4 h. This suggested that the degradation of EM took place during the shaking. The t_R of the unknown peak on the HPLC chromatogram was 4.5 min. This peak had a very weak fluorescence on excitation at 314 nm with emission at 424 nm. Increase in the shaking time caused an increase of the height of the peak, and its peak

height ratios relative to IS were 0.019 at 30 min and 0.049 at 60 min. This finding suggests that the degradation of EM cannot be detected by the colorimetric method. Therefore, the procedure of the content uniformity test and the colorimetric method for determination of EM tablets in JP X should be changed.

References and Notes

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