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REVERSED-PHASE DETERMINATION OF FAMOTIDINE, POTENTIAL DEGRADATES, AND PRESERVATIVES IN PHARMACEUTICAL FORMULATIONS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY USING SILICA AS A STATIONARY PHASE

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SUMMARY

An analytical method was developed for the determination of famotidine, potential degradates, and preservatives in several pharmaceutical formulations. The method employs reversed-phase chromatography on a silica column with a methanol–phosphate solution as the mobile phase. The influence of the concentration of phosphate and organic modifier are discussed. Accuracy and precision for this method along with assay data from different formulations of famotidine are presented.

Two different commercial silica columns were tested with this method. Chromatographic differences related to the surface area and pore size of the silica are discussed.

INTRODUCTION

Famotidine (Fig. 1), a new H_2-receptor antagonist, is a highly potent inhibitor of gastric acid secretion in humans[1,2]. Several formulations of famotidine have been developed for clinical evaluation, including an oral-dosage powder for reconstitution formulation containing the preservatives benzoic acid, methylparaben and propylparaben, a parenteral formulation containing benzyl alcohol as a preservative and a tablet formulation.

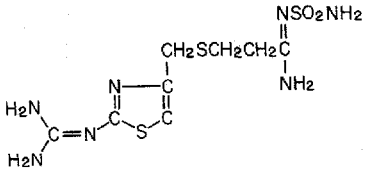

Fig. 1. Chemical structure of famotidine, 3{[([2-(diaminoethylene)amino]-4-thiazolyl)methyl]thio}-N-sulfamoyl-propionamide.

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A sensitive and specific high-performance liquid chromatography (HPLC) procedure was developed for the quantification of famotidine, its potential degradates and the preservatives using reversed-phase HPLC on silica[3–5]. This alternative to reversed-phase HPLC on chemically-modified silica stationary phases produced an excellent separation that could be used for both clinical release and stability testing. This method evolved due to poor and variable chromatography of the oral-dosage formulation using a previously reported method on a chemically bonded stationary phase[6]. Using a silica stationary phase, reproducibility and selectivity were greatly improved and column-to-column variation was minimized.

Silica columns from two different manufacturers with different surface areas were evaluated. Although selectivity was identical, the resolution of the preservatives was related to the surface area of the silica. The sodium dihydrogen phosphate concentration of the eluent required to produce similar retention characteristics for famotidine on the silica substrates tested was also found to be related to the surface area of the silica.

EXPERIMENTAL

Reagents

Water was purified with a Milli-Q-Water System (Millipore, Bedford, MA, U.S.A.). Methanol was Fisher HPLC-grade (Fisher Scientific, King of Prussia, PA, U.S.A.). Sodium phosphate monobasic (AR grade) was from Mallinckrodt (St. Louis, MO, U.S.A.). Reference standards benzoic acid, methylparaben, propylparaben and benzyl alcohol were obtained from the USP (Rockville, MD, U.S.A.). A famotidine reference standard as well as potential degradates A_1, A_2 and A_3 reference standards were obtained from Merck Sharp and Dohme Research Laboratories (West Point, PA, U.S.A.).

Apparatus

The HPLC system consisted of a Hewlett-Packard (Avondale, PA, U.S.A.) Model 1090 pump module, with a Kratos (Ramsey, NJ, U.S.A.) Model 757 variable-wavelength detector operated at 254 nm and 0.01 a.u.f.s. Chromatograms were collected and integrated using a Hewlett-Packard Model 3390A integrator or a Hewlett-Packard Model 3357B laboratory automation system.

Chromatography

The analytical column was a Beckman (Altex Scientific, Berkeley, CA, U.S.A.) Ultrasphere silica column (25 cm × 4.6 mm I.D., 5 μm particle size). The mobile phase was methanol–water–0.05 *M* sodium dihydrogen phosphate (10:74:16, v/v/v). The mobile phase was filtered through a 0.45-μm Nylon-66 membrane. The flow-rate was 1.0 ml/min. All chromatography was performed at room temperature (23–26°C) using 25-μl injections. A Dupont (Wilmington, DE, U.S.A.) Zorbax silica column (25 cm × 4.6 mm I.D., 5 μm particle size) was also employed using appropriate mobile phase composition modifications.

Sample preparation

The parenteral solution formulation was diluted with mobile phase to give a final famotidine concentration of 0.08 mg/ml.

Tablet formulations were ground and an accurately weighed portion of the powder equivalent to 8 mg of famotidine was dissolved and diluted to 100 ml with the mobile phase.

An accurately weighed portion of the oral-dosage powder for reconstitution formulation equivalent to 8 mg of famotidine was dissolved and diluted to 100 ml with the mobile phase. Samples were filtered through a 0.45-μm Nylon-66 membrane and 25 μl of each sample was injected into the HPLC system.

Standard preparation

Standard solutions were prepared by dissolving famotidine reference standard and benzyl alcohol or benzoic acid, methylparaben and propylparaben in the mobile phase to obtain concentrations of 0.08 mg/ml, 0.07 mg/ml, 0.05 mg/ml, 0.05 mg/ml and 0.02 mg/ml, respectively. Potential degradates A_1, A_2 and A_3 were added at the 1% (w/w) level to the famotidine standard solution, when appropriate. Sample concentrations were calculated by comparing HPLC peak areas with the area of the appropriate standard peak.

RESULTS AND DISCUSSION

With the described HPLC procedure famotidine is separated from its potential degradates and the preservatives contained in the pharmaceutical formulations. A typical chromatogram of the oral-dosage powder for reconstitution formulation that was spiked with 1.0% (w/w) of three possible degradates of famotidine is shown in Fig. 2. The selectivity for this method was adjusted in order to separate these impurities. A chromatogram of the parenteral solution containing benzyl alcohol is shown in Fig. 3.

The retention of famotidine and the preservatives is affected by the concentration of methanol and sodium dihydrogen phosphate in the mobile phase. The influ-

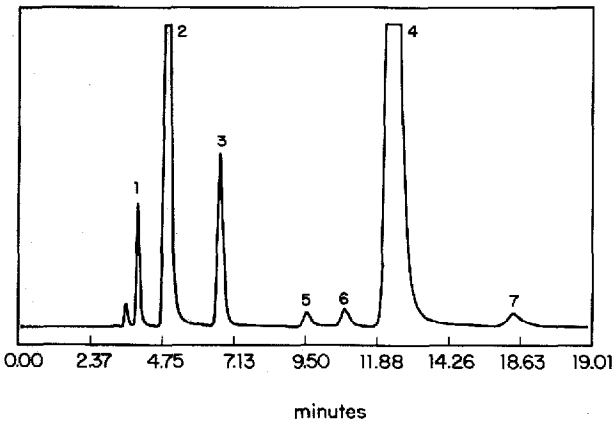

Fig. 2. Chromatogram of famotidine in an oral-dosage powder for reconstitution formulation spiked with 1.0% (w/w) of three possible impurities. Column, Ultrasphere silica (250 × 4.6 mm I.D.); mobile phase, methanol–water–0.05 *M* sodium dihydrogen phosphate solution (10:74:16); flow-rate, 1.0 ml/min; peaks: 1 = benzoic acid, 2 = methylparaben, 3 = propylparaben, 4 = famotidine, 5, 6, 7 = famotidine potential degradates A_2, A_1 and A_3, respectively.

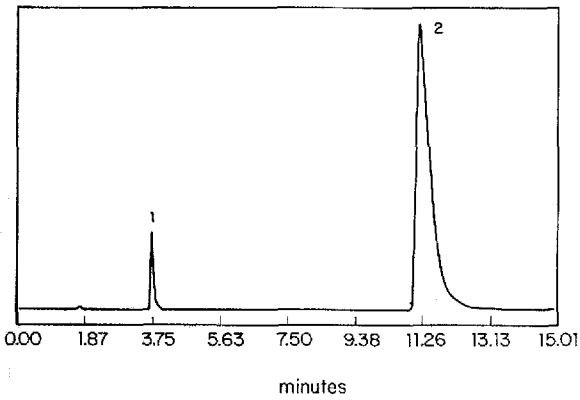

Fig. 3. Chromatogram of famotidine in a parenteral solution formulation. Column, Ultrasphere silica (250 × 4.6 mm I.D.); mobile phase, methanol–water–0.05 *M* phosphate solution (10:74:16); flow-rate, 1.0 ml/min; peaks: 1 = benzyl alcohol and 2 = famotidine.

ence of the ionic strength on retention was investigated by varying the sodium dihydrogen phosphate concentration. Table I shows the retention of benzoic acid, the paraben preservatives and famotidine with various concentrations of phosphate salt in the mobile phase. The decrease in retention of famotidine with increasing sodium dihydrogen phosphate concentration (*i.e.* ionic strength) is postulated to be either due to the competing-ion effect of sodium[3] or an ion-exchange effect[5]. Sodium ions preferentially compete with the cationic famotidine for available $Si–O^-$ sites in this system with larger concentrations of sodium ion decreasing the sites available for interaction between famotidine and the silica stationary phase. The retention of the preservatives, however, is unaffected by ionic strength with the exception of benzoic acid which slightly increases in retention due to a decrease in pH with increasing phosphate concentration. Since the preservatives are essentially unaffected by changes in ionic strength, the elution characteristics of these compounds can be optimized by varying the amount of methanol in the mobile phase. Table II shows the retention of the preservatives and famotidine with various concentrations of meth-

TABLE I

INFLUENCE OF PHOSPHATE CONCENTRATION IN THE MOBILE PHASE ON RETENTION TIME

Eluent: methanol–water (10:90) plus phosphate at the stated concentrations.

Phosphate concentration (mM)	*Retention time (min)*			
	Benzoic acid	*Methyl paraben*	*Propyl paraben*	*Famotidine*
6	2.72	3.55	4.79	13.86
8	2.79	3.55	4.82	12.07
10	2.84	3.54	4.76	10.72
12	2.89	3.53	4.72	9.84

TABLE II

INFLUENCE OF METHANOL CONCENTRATION IN THE MOBILE PHASE ON RETENTION TIME

Eluent: 8 m*M* phosphate in water (pH 4.6) containing the stated concentration of methanol

Methanol concentration (%)	*Retention time (min)*			
	Benzoic acid	*Methyl paraben*	*Propyl paraben*	*Famotidine*
6	2.84	3.70	5.52	14.60
8	2.79	3.55	4.82	12.07
10	2.78	3.44	4.36	10.10
12	2.76	3.35	4.05	8.98

anol. The retention times of all the components decrease with increasing methanol concentration as is expected when a "reversed-phase" retention mechanism is at work. Based upon the effects of changing ionic strength and methanol concentration in the mobile phase, it is postulated that retention of famotidine is due to a mixed-mode mechanism governed by ion-exchange and reversed-phase phenomena, whereas the retention of the preservatives is predominantly due to a reversed-phase mechanism.

The repeatability of the chromatographic system was shown by chromatographing ten injections of the standard solutions for the parenteral formulation and the oral-dosage powder for reconstitution formulation. The relative standard deviations of the peak areas for benzyl alcohol, benzoic acid, methylparaben, propylparaben and famotidine were 0.52%, 1.10%, 0.47%, 0.36% and 0.45%, respectively.

The accuracy of the method for each of the five components was determined by a three concentration level recovery study of the components from spiked placebos of the parenteral and oral-dosage powder for reconstitution formulations. The recovery (%) (± relative standard deviation) for benzyl alcohol, benzoic acid, methylparaben, propylparaben and famotidine was 100.1 ± 0.7%, 100.5 ± 0.6%, 100.5 ± 0.5%, 99.5 ± 0.8% and 99.7 ± 0.5%, respectively. A similar recovery experiment from a famotidine tablet placebo produced a result of 99.8 ± 0.4% for famotidine. A standard famotidine solution was serially diluted and assayed to obtain the limit of detection for this method. A level of 50 ng/ml (1.25 ng injected) was found to be the detection limit[2] for famotidine.

Ten identical samples of the oral-dosage powder for reconstitution formulation were assayed to determine the assay precision for each component. The assay precision expressed as the relative standard deviation for benzoic acid, methylparaben, propylparaben and famotidine was ±0.81%, ±0.62%, ±0.84% and ±0.36%, respectively.

Three lots encompassing the various formulations of famotidine were analyzed by this method. The results for 40 mg oral-dosage powder for reconstitution, 10 mg/ml preserved parenteral and 20 mg tablet formulations are shown in Table III. The level of famotidine varied between 97.4% and 100.5% of the label claim amount for the various formulations. Acceptable results for all analytes indicate that this

TABLE III

FAMOTIDINE FORMULATIONS: HPLC ASSAY RESULTS

The assay value given is the mean ± S.D.

Formulation and potency claim	*Famotidine (mg)*	*Potency claim for each component (%)*	*Benzoic acid (mg)*	*Claim (%)*	*Methylparaben (mg)*	*Claim (%)*	*Propylparaben (mg)*	*Claim (%)*	*Benzyl alcohol (mg)*	*Claim (%)*
40 mg oral-dosage	39.0 ± 0.15	97.4	5.1 ± 0.18	101.4	4.8 ± 0.14	95.7	1.0 ± 0.04	104.2		
10 mg/ml preserved parenteral	10.1 ± 0.06	100.5							9.1 ± 0.10	101.4
20 mg tablet	19.9 ± 0.45	99.3								

method is suitable for use in clinical release and/or stability testing for all famotidine pharmaceutical formulations.

The 5-μm Ultrasphere silica used for this procedure is reported[7] to have an average pore diameter of 9 nm and a surface area of 200 m^2/g. These silica characteristics provided for acceptable peak shape and resolution for the components contained in the various dosage forms. A second supply of silica (Dupont Zorbax Sil, 5 μm) was evaluated, having an average pore diameter of 8 nm and a surface area of 350 m^2/g (ref. 8). With this silica column the capacity factor (k') of the propylparaben peak increased by more than a factor of three using the same mobile phase as that used for the Ultrasphere silica column. In order to elute famotidine from the Zorbax silica with a k' similar to that obtained with the Ultrasphere silica, the phosphate concentration in the eluent had to be increased from 8 mM to 25 mM. As a result of the increased retention characteristics of the Zorbax silica, methanol–water–0.05 M phosphate solution (24:70:6) had to be used as the eluent in order to produce a chromatogram similar to that produced with the Ultrasphere silica. These results imply that the increased surface area of Zorbax silica apparently produces a greater number of siloxane and silanol sites available for interaction with the analytes. Bidlingmeyer *et al.*[5] reported a similar relationship between silica surface area and retention phenomena.

CONCLUSION

Silica columns can be operated in a reversed-phase mode using an aqueous sodium dihydrogen phosphate solution with an organic modifier as the mobile phase. Two different silica packings were tested for retention characteristics using this method. Although selectivity for the component peaks was the same, the degree of retention was related to the surface area of the silica. The use of an aqueous reversed-phase eluent on silica represents a viable alternative to traditional reversed-phase HPLC on chemically-bonded phases. Using the described method, retention of the analytes is controlled by adjusting the concentration of organic modifier in the mobile phase. The retention of cationic analytes is also affected by the ionic strength of the mobile phase. The described method has been shown to be accurate, specific for the analytes, precise, and rugged for routine clinical release and stability testing of several pharmaceutical formulations of famotidine.

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