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Peak distortion in the column liquid chromatographic determination of omeprazole dissolved in borax buffer☆

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ABSTRACT

Injection of a sample containing omeprazole dissolved in borax buffer (pH 9.2) into a reversed-phase liquid chromatographic system consisting of a mixture of acetonitrile and phosphate buffer (pH 7.6) as the mobile phase and a C_{18} surface-modified silica as the solid phase resulted under special conditions in split peaks of omeprazole. The degree of peak split and the retention time of omeprazole varied with the concentration of borax in the sample solution and the ionic strength of the mobile phase buffer as well as with the column used. Borax is eluted from the column in a broad zone starting from the void volume of the column. The retention is probably due to the presence of polyborate ions. The size of the zone varies with the concentration of borax in the sample injected. In the borax zone the pH is increased compared with the pH of the mobile phase, and when omeprazole (a weak acid) is co-eluting in the borax zone its retention is affected. In the front part and in the back part of the borax zone, pH gradients are formed, and these gradients can induce the peak splitting. When the dissolving medium is changed to a phosphate buffer or an ammonium buffer at pH 9 no peak distortion of omeprazole is observed.

INTRODUCTION

Omeprazole is a substituted benzimidazole which selectively inhibits the proton pump in the gastric mucosa [1]. In solution omeprazole degrades rapidly at low pH values [2]. Therefore, during analysis omeprazole is preferably dissolved under alkaline conditions. Omeprazole can be dissolved in a borax buffer of pH 9.2 and assayed by reversed-phase chromatography with a C_{18} modified silica as the solid phase and with a phosphate buffer–acetonitrile mixture as eluent. However, in some cases, injection of such a sample into the liquid chromatographic system results in a deformed or even split peak of omeprazole.

Peak deformation is a commonly occurring phenomenon in column liquid chromatography. Sample overloading often results in peak tailing. For hydrophobic amines severe peak tailing is obtained even at low sample concentrations, which can be explained as being due to a heterogeneous column surface [3], an effect often named 'the silanol effect' [4]. Most peak distortion phenomena can be related to disturbances in the column equilibrium. In ion-pairing systems split, broadened and compressed peaks of the analyte have all been observed. These occur when the sample injected has a different concentration of the ion-pairing reagent compared with the mobile phase, or contains a second non-detectable hydrophobic ion. The phenomena have been explained as being due to co-elution of the analyte

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in a non-detectable zone induced by the ion-pairing reagent [5,6]. Also, in a simple chromatographic system containing buffer and acetonitrile as eluent, similar effects have been obtained when a hydrophobic non-detectable ion is injected and co-eluted with the analyte [7].

Deformed peaks have also been reported when applying samples with the ability to form strong complexes between the analyte and an agent in the sample solution in cases when the analyte and the complexing agent have different retentions [8]. The strong drug–protein binding between naproxen and albumin results in a deformed peak of naproxen [8]. Adduct formation between tannins and methanol in the sample solution results in deformed peaks of the tannins [9].

Peak splitting has also been observed in chromatography of proteins when the protein occurs in both native and denaturated forms. Two separate peaks are obtained [10].

In the present study deformed peaks of omeprazole in borax buffer were observed. The degree of peak distortion varied from column to column and with the concentration of borax in the sample solution. This study attempts to explain this phenomenon.

EXPERIMENTAL

Chemicals

Omeprazole was obtained from Hässle (Mölndal, Sweden). Sodium tetraborate ($Na_2B_4O_7 \cdot 10H_2O$, borax) was obtained from Merck (Darmstadt, Germany). Acetonitrile was of high-performance liquid chromatography (HPLC) grade and was obtained from Merck or from Rathburn (Walkerburn, UK). All other chemicals were of analytical grade.

Equipment

The pump was a LKB 2150 (LKB, Bromma, Sweden) or an SP 8700 (Spectra Physics, San José, CA, USA). The injector was a WISP 710B, (Waters Assoc, Milford, MA USA) or an SP 8780XR (Spectra Physics). The UV detector was a Spectraflow 783 (Kratos, Ramsey, NJ, USA). UV spectra were obtained with a 1000S diode-array detector (Applied Biosystems, Ramsey, NJ, USA). The integrator was an SP 4270 (Spectra Physics).

The columns were 150 × 4.6 mm I.D. Nucleosil C_{18}, 5 μm (Macherey-Nagel, Duren, Germany) or 100 × 5 mm I.D. Novapak C_{18}, 4 μm, Radial PAK (Waters Assoc.) fixed in a Waters RCM 8 × 10 cartridge holder.

A micro combined pH-electrode with needle membrane (Ingold, Urdert, Switzerland) was used to measure the pH of the column elutate.

Procedures

Stock solutions of omeprazole were prepared by dissolving 5 mg of omeprazole in 5 ml of ethanol followed by dilution to 25 ml with 0.01 *M* borax solution. Sample solutions were obtained by diluting the stock solution with aqueous borax solutions.

The phosphate buffers were prepared by mixing different volumes of 1 *M* phosphoric acid, 1 *M* sodium dihydrogenphosphate, 0.5 *M* disodium hydrogenphosphate and 0.25 *M* trisodium phosphate, which were diluted with water to obtain the specified pH and ionic strength. The eluents were prepared by mixing volumes of buffer and acetonitrile in the specified ratios.

A 10-to 20-μl aliquot of the sample was injected into the column with a flow-rate of 1.0 ml/min. Detection of omeprazole was made at 280 nm and of borax at 190 nm. The conditional pH (pH*) of the column eluate was measured in 0.5-ml fractions.

Computer calculations

Calculation of polyborate equilibria was made with a personal computer using the programs INPUT and SED obtained from the Department of Inorganic Chemistry, KTH (Stockholm, Sweden). The programs are based on the well known programs HALTAFALL [11] and SOLGASWATER [12].

RESULTS AND DISCUSSION

Chromatography of omeprazole

Omeprazole (Fig. 1) is an ampholyte with pK_a of about 4 (pyridinium) and 8.8 (benzimidazole). It is rapidly degraded in acidic solutions but has an acceptable stability at higher pH. The half-life of omeprazole in a solution of pH 6.5 is about 18 h and at pH 11 about 300 days [2]. It is therefore suitable to have sample solutions at high pH during the chromatographic analysis and to perform the chromato-

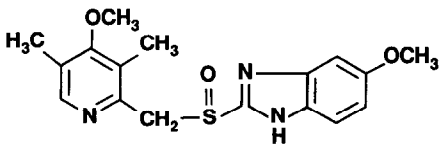

Fig. 1. Chemical structure of omeprazole.

graphy with a mobile phase of a pH as high as possible.

A phosphate buffer of pH 7.5 with an ionic strength (μ) of 0.025 was chosen as mobile phase buffer. Because of the risk of degradation of the silica-based column, a higher pH was avoided. The acetonitrile concentration varied between 25 and 40% depending on the column used. The retention of omeprazole is sensitive to changes in pH above 7.5, which is demonstrated in Table I, which shows the effect of using phosphate buffers of various pH as the mobile phase with acetonitrile. This is obviously due to the protolysis of the molecule, which should be noticeable in this pH range.

To avoid degradation, omeprazole was dissolved in a sodium tetraborate solution of pH 9.2. This means that the solvent of the sample differs widely from that of the mobile phase.

A distorted peak of omeprazole was found on some columns (Fig. 2, upper chromatograms). Different batches of columns from the same brand gave different results. When the distortion occurred the omeprazole peak was usually split into two peaks, one large peak eluting before a smaller second peak (Fig. 2A, I). The degree of peak split increased with increasing borax concentration in the sample solution (Fig. 2A and B).

The distortion was found to be due to the borax buffer since, when omeprazole was dissolved in other types of buffers (ammonium or phosphate) with the same pH as the borax buffer or in the eluent, a normal peak shape of omeprazole was obtained, *i.e.* similar to the peak shown in Fig. 2B, I.

TABLE I

INFLUENCE OF THE MOBILE PHASE pH ON THE RETENTION OF OMEPRAZOLE

Eluent: acetonitrile–phosphate buffer ($\mu = 0.025$) (40:60, v/v)
Column: Nucleosil C_{18}

Mobile phase pH	k' omeprazole
6.5	2.0
7.5	2.0
8.5	1.1

Retention of borax buffer

To study the influence of borax buffer in the sample solution, samples of omeprazole with different concentration of borax buffer were injected. Omeprazole was detected by UV at 280 nm, whereas borax was detected by UV at 190 nm. The results from three different columns (Fig. 2) show that the borax buffer was retained as a wide zone. The shape of the zone varied drastically from column to column, and the width of the zone increased with the borax concentration (columns A and B).

Retention of omeprazole in borax zone

The retention of omeprazole decreases with increasing borax concentration in the sample solution. This is illustrated by using column C (Fig. 2C) where omeprazole is eluted within the borax zone and the peak is not split, *i.e.* omeprazole has a lower retention in the borax zone than in the bulk mobile phase.

In columns A and B (Fig. 2A and B) the retention of omeprazole also decreased with increasing borax concentration in the sample solution, but peak split occurred. A small peak containing omeprazole with the same retention as if omeprazole were dissolved in the bulk mobile phase occurs. The retention of the rest of omeprazole, the split part, decreases with increasing borax concentration. In column B (Fig. 2B, III), omeprazole is even split into three peaks. In the lower chromatogram in Fig. 2A (III) when the injected sample contained 0.050 *M* borax, the first eluting peak of omeprazole was compressed, which seems to be because of the elution of omeprazole in a gradient.

In column C (Fig. 2C) omeprazole is not split. This may be because omeprazole is eluted within the borax zone and no steep gradient of the borax zone is present.

pH in the borax zone

The results above clearly show that the retention of omeprazole is affected by the borax buffer. The retention of omeprazole is dependent on the mobile phase pH (Table I). Therefore, experiments were

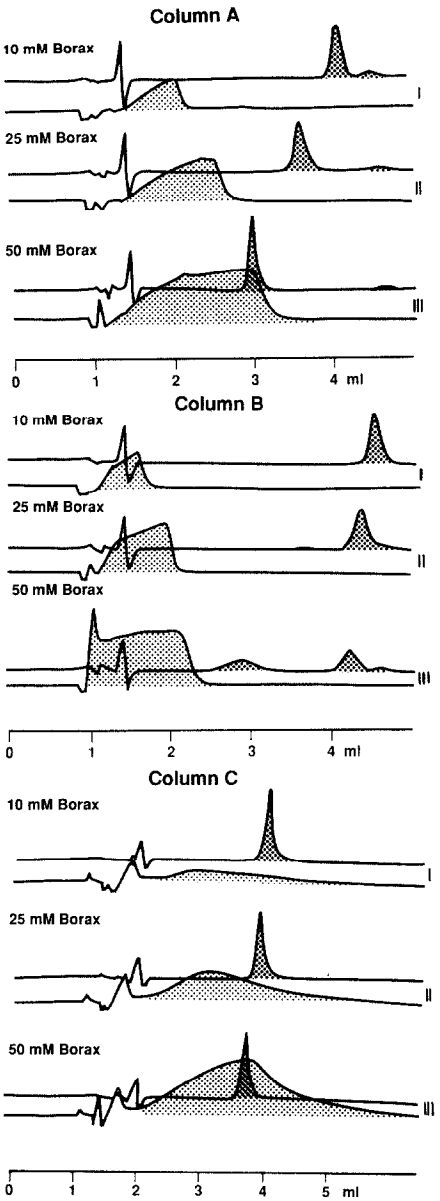

Fig. 2. Retention of omeprazole and borax in borax buffer. Samples: 1 μM omeprazole-containing borax and borax. Upper (I) 10 mM borax, middle (II) 25 mM borax, lower (III) 50 mM borax. Detection: omeprazole (darker shaded peak) UV at 280 nm, borax (lighter shaded peak) UV at 190 nm. Columns: A and B Novapak C_{18}, C Nucleosil C_{18}. Eluent: acetonitrile–phosphate buffer pH 7.6 ($\mu = 0.025$): A and B 30:70 (v/v); C 40:60 (v/v).

performed to determine the pH of the eluate. Fractions of the eluate (0.5 ml) were collected and the conditional pH (pH*) was determined with a microelectrode. The results after injection of a 0.1 M borax solution showed (Fig. 3) that the pH* was increased by about 0.5 units in the borax zone compared with the pH* in the bulk mobile phase and that pH gradients were formed in the front and back parts of the borax zone. Changing the mobile phase pH by 0.5 units drastically influences the retention of omeprazole (Table I). The peak-split phenomenon may be explained by the pH difference between the sample solution and the mobile phase. The plug created by injection of borax gives pH gradients in the front and back parts of the zone. When omeprazole is retained in the steep back gradient of the borax zone the back part of the omeprazole zone has a lower retention compared with the front part, whereas in the front gradient the situation is reversed, *i.e.* the back part of the omeprazole zone has a higher retention than the front part.

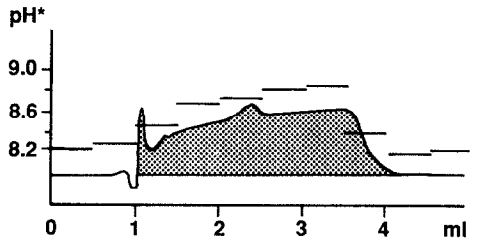

Fig. 3. pH of eluate after borax buffer injection. Sample: 10 μl of 100 mM borax. Detection: UV at 190 nm (shaded peak). pH* of 0.5 ml fractions are indicated by horizontal bars. Column: Novapak C_{18} (RCM). Eluent: acetonitrile–phosphate buffer pH 7.5 ($\mu = 0.025$) (30:70, v/v)

Therefore, around the point where the gradients meet the omeprazole molecules will struggle in different directions, *i.e.* they will have different migration rates depending on the gradient in which they are eluted, and in that way induce peak splitting.

At high borax concentrations (Fig. 2B, III) the borax zone seems to create several pH gradients and omeprazole is split into three peaks.

Identity of omeprazole

The identity of omeprazole in the split peaks was examined using a photo diode-array detector, and agreement between the spectra from the two peaks was obtained (Fig. 4).

Borax buffer

When dissolving borax in water, boric acid and

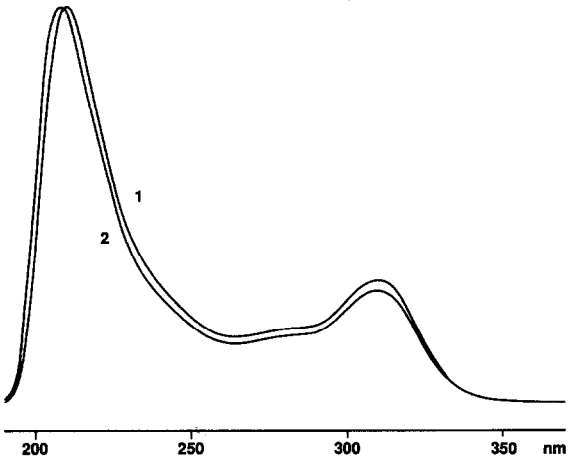

Fig. 4. Photodiode-array UV spectra from split omeprazole peaks. Normalized absorbances from peak maximum. Chromatogram similar to Fig. 2 (column A, I). 1 = Large peak; 2 = small peak.

borate ions are formed. The pK_a value is about 9.2. However, according to Ingri [13], at higher concentrations of borate, polyborate ion complexes can be formed. Stability constants for the different borate polyions were determined [13]. The distribution of boron between different ions was calculated from the stability constants given by Ingri [13] for three different borax concentrations and is presented in Fig. 5. The figure shows that the fraction of polyions increases with increasing borate concentration. It also shows that polyions occur at pH between 6 and 11, *i.e.* at the pH used in the mobile phase for the omeprazole assay polyions certainly exist. When injecting borax buffer into a chromatographic system consisting of a mobile phase of acetonitrile–phosphate buffer, pH 3.0, or a similar phase with pH 11.0, only one peak eluting with the front was observed. This indicates that both boric acid and the borate ion are eluted unretained. The retention of borax seems to be affected by the polyanions.

The column equilibrium is obviously attributed to the distribution between the different polyions, which varies with the borax concentration (see Fig. 5), *i.e.* with the dilution of the injected zone during chromatographic elution. Since the polyborates are retained and boric acid as well as borate are unretained, the retention mechanism will be very complex.

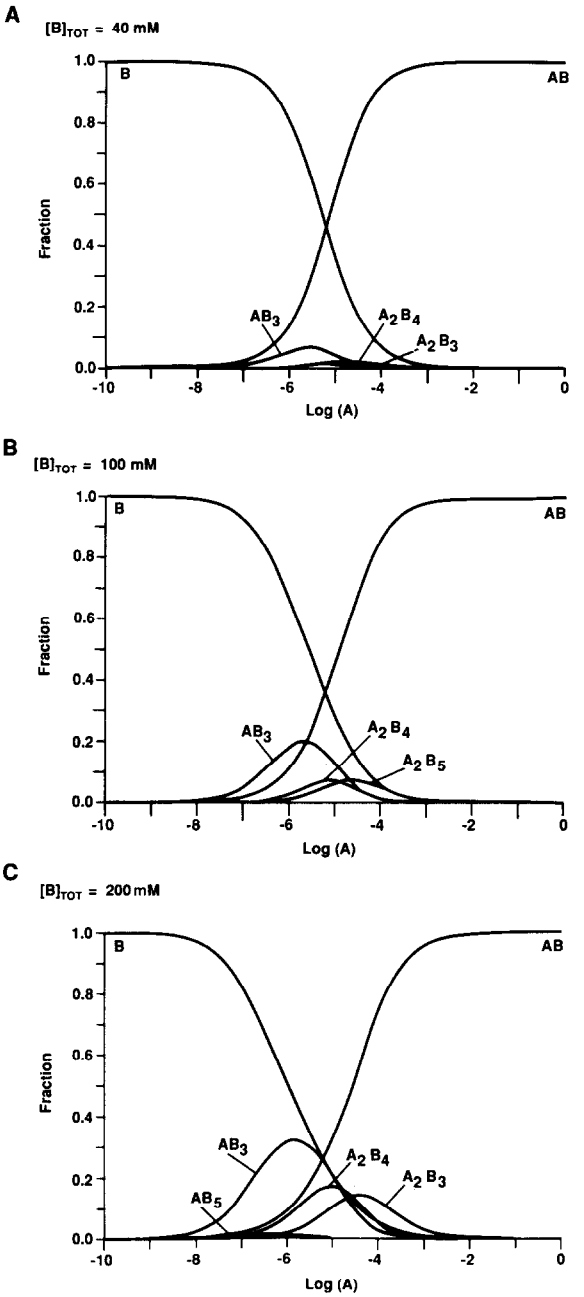

Fig. 5. Distribution of boron between different ions in borax solution: (A) 10 mM borax; (B) 25 mM borax; (C) 50 mM borax. Calculated by computer programs with stability constants according to Ingri [13]. A = $[OH^-]$, B = $[B(OH)_3]$ and $\beta_{pq} = [A_pB_q[A]^{-p}[B]^{-q}$. $\log \beta_1 = 5.2$, $\log \beta_{13} = 7.3$, $\log \beta_{24} = 13.5$, $\log \beta_{23} = 12.0$ and $\log \beta_{15} = 7.4$.

Effects of the ionic strength in the mobile phase

In the study so far the mobile phase buffer ionic strength was 0.025. The buffer capacity of the mobile phase may be too low to neutralize the sample zone containing borax efficiently. If the mobile phase ionic strength is increased, the pH of the borax zone may decrease, the pH gradients will be reduced and the influence of the polyborates will become negligible as their concentration approaches zero (see Fig. 5). The results (Fig. 6) show that the peak distortion of omeprazole decreases with increasing ionic strength. The peak split disappeared when using an ionic strength of 0.1 in the eluent. The retention of omeprazole as well as the size of the borax zone decreases with increasing ionic strength. The effect is possibly due to the fact that the pH in the zone decreases, thus counteracting the formation of the polyborate ions.

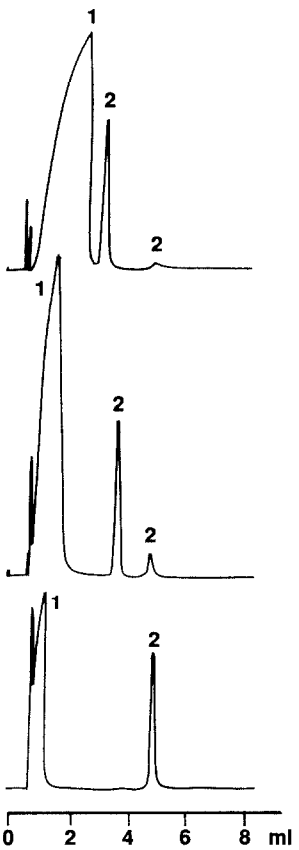

Fig. 6. Effect of mobile phase pH ionic strength on peak split of omeprazole. Sample: 1 μM omeprazole in 50 mM borax. Detection: UV at 190 nm. Peaks: 1 = Borax; 2 = Omeprazole. Eluent: acetonitrile–phosphate buffer pH 7.6 (30:70, v/v). Ionic strength of buffers: upper chromatogram, 0.025; middle chromatogram, 0.05; lower chromatogram, 0.10. Column: Novapak C_{18} (RCM).

CONCLUSIONS

Sample solutions containing borax buffer may give peak disturbances of the analyte. The effect occurs predominantly when the pH of the mobile phase is 6–8. The reason for the disturbance was found to be the presence of polyborate ions, which were retained as a broad zone. In this zone the pH was increased compared with the bulk mobile phase and pH gradients were created. Disturbance occurred when the analyte was present in the pH gradient and its retention was affected by a change of the pH. It was possible to decrease the disturbances by increasing the ionic strength of the mobile phase. If the sample was dissolved in other types of buffer, *e.g.* phosphate or ammonium, no peak distortion of omeprazole was observed.

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