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# Kinetics of Aspirin Absorption Following Oral Administration of Six Aqueous Solutions with Different Buffer Capacities

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Received October 29, 1982, from the *Pharmacokinetics Laboratory, Schools of Pharmacy and Medicine, University of Missouri-Kansas City, Kansas City, MO 64108.* Accepted for publication September 13, 1983.

**Abstract** □ Fifteen volunteers each received two of six aspirin solutions in a balanced incomplete block design. The solutions contained 0, 3, 6, 10, 16, and 34 mEq of sodium bicarbonate-citric acid buffer and 650 mg of aspirin. Plasma aspirin levels were measured in blood samples collected frequently during the first 2.5 h, and the accumulation of aspirin, salicylic acid, and salicyluric acid were measured over 2 and 24 h. The most rapid absorption rates occurred with solutions which contained small quantities of the antacid buffer. The 3- and 6-mEq antacid buffers had mean maximal aspirin concentrations of 17.3 and 17.8 μg/mL, respectively. In the absence of the buffering agent, the 650 mg of aspirin failed to dissolve completely and gave a mean maximal plasma concentration of 13.4 μg/mL. With 34 mEq of a buffering agent, a delay in the onset of absorption occurred and the presystemic hydrolysis increased. This was probably because more aspirin was emptied into and absorbed from the small intestine with higher concentrations of the buffering agent.

**Keyphrases** □ Aspirin—pharmacokinetics, oral administration, sodium bicarbonate-citric acid buffer, humans □ Pharmacokinetics—aspirin, sodium bicarbonate-citric acid buffer, oral administration, humans

Aspirin, the drug of choice when a mild analgesic-antipyretic is required, is also the primary agent in the chronic management of rheumatoid arthritis and osteoarthritis. After oral administration, rapid absorption beneficially provides rapid onset of effects and reduces the contact time with the gastric mucosa.

In an earlier report from this laboratory (1) the literature relating to the administration of aspirin in buffered solutions was reviewed, and data comparing an unbuffered tablet to two different buffered solutions was presented. The three dosage forms were an unbuffered tablet, an effervescent solution with 16 mEq of buffer, and an effervescent solution with 34 mEq of buffer. Significant differences in the aspirin absorption rate were observed: the solution of 16 mEq of buffer was fast, the solution of 34 mEq of buffer was intermediate, and the unbuffered tablet was slow. These differences were attributed primarily to gastric emptying and the rate of tablet dissolution.

It may be desirable to optimize the amount of buffer components to provide rapid absorption and to reduce gastric irritation through minimal alkalinization of urine and sodium intake. Thus, this study was undertaken to evaluate aspirin absorption kinetics over a range of buffer strengths.

## EXPERIMENTAL SECTION

**Dosage Forms**—A series of soluble tablets were prepared that contained 650 mg of aspirin and sufficient sodium bicarbonate-citric acid buffer to provide 0, 3, 6, 10, 16, and 34 mEq of buffering. Just prior to administration, the tablets were placed in 140 mL of water and mechanically stirred to effect dissolution. The unbuffered tablet contained some undissolved particles in suspension, while all buffered tablets dissolved completely. Immediately following administration, the containers were rinsed with 100 mL of water which was also swallowed by the volunteers.

**Subjects**—Fifteen healthy volunteers, 21-33 years old and weighing 56.4-81.8 kg, were screened by a comprehensive physical examination, complete blood chemistry, complete blood count with differential, and urinalysis. All were free of any active disease such as influenza, and denied any use of medication for 14 d prior to the study. None of the subjects had a history of GI disease or surgery.

**Method**—The balanced incomplete block design, Table I, in which each of the 15 subjects received two of the six dosage forms with a 6-d washout between treatments, was employed. A 10-h fast preceeded dosing and continued 4 h postdose, except for water. Predose urine and blood samples were obtained, and a single dose of aspirin (650 mg) with 240 mL of water was administered as described. After dosing, 100 mL of water was administered at 1-, 2-, and 3-h intervals, and a uniform meal was served after 4 h. Subjects remained standing or sitting throughout the first 4 h, and exercise was limited to walking about the room.

Blood was drawn into chilled vacuum containers[1] *via* an indwelling catheter at 5, 10, 15, 20, 30, and 45 min and at 1, 1.5, 2, and 2.5 h. Plasma was separated by centrifugation at 1764×g within 20 min of collection. All urine was collected over a 0-2-h interval, the pH and volume were measured, and a 4-mL aliquot was taken for analysis. In addition, all urine was collected for 24 h. All plasma and urine samples were handled as described previously (2), which included storage at −20°C and HPLC analysis within 2 weeks. The HPLC method was updated by using a 5-μm octadecasilane column[2], measuring absorbance at 237 nm, and replacing the benzene with butyl chloride. These changes improved the sensitivity, safety, and reproducibility of the method over that reported previously (2); the statistics of the revised method will be the subject of a subsequent report.

Plasma aspirin concentration *versus* time plots for each dose to each subject were evaluated, and estimates for several kinetic parameters were obtained. The highest observed plasma concentration ($C_{max}$) and the time after dosing ($t_{max}$) to reach $C_{max}$ were read directly from each plot. A terminal rate constant ($k$) and corresponding half-life ($t_{1/2}$) were computed by linear regression analysis of the natural logarithm of plasma concentration *versus* the time for all times greater than twice $t_{max}$. The trapezoid method was used to compute the area under the curve to the last measured concentration ($C$) and the value

[1] Vacutainer BD, 278-069, 7.0-mL capacity containing 14 mg of potassium oxalate and 17.5 mg of sodium fluoride.
[2] Altex Ultrasphere, ODS 5 μm; Altex Scientific Inc., Berkeley, Calif.



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**Table I—Balanced Incomplete Block Design**

| Subject Number | Antacid Buffering Agent, mEq | |
|---|---|---|
| | 1st Dose | 2nd Dose |
| 1 | 16 | 6 |
| 2 | 3 | 16 |
| 3 | 34 | 10 |
| 4 | 10 | 6 |
| 5 | 6 | 0 |
| 6 | 0 | 16 |
| 7 | 0 | 34 |
| 8 | 34 | 3 |
| 9 | 16 | 34 |
| 10 | 10 | 0 |
| 11 | 16 | 10 |
| 12 | 6 | 3 |
| 13 | 34 | 6 |
| 14 | 3 | 0 |
| 15 | 10 | 3 |

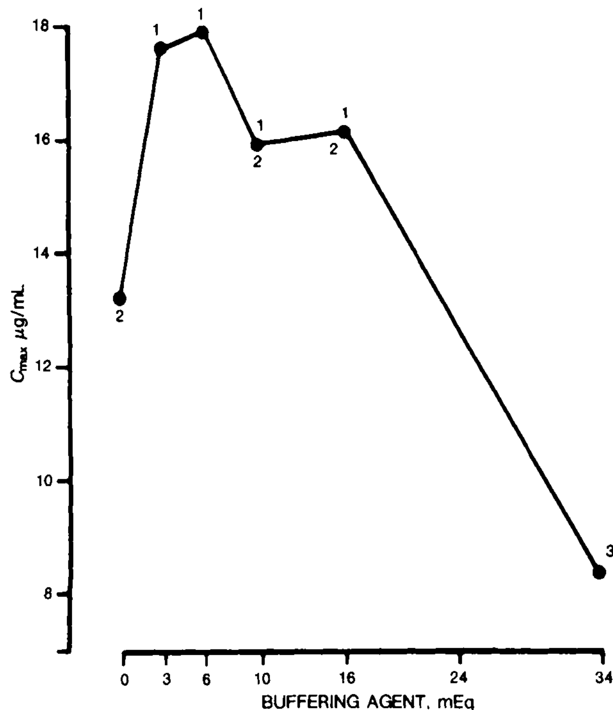


**Figure 1**—*A plot of $C_{max}$ for aspirin as a function of buffer strength. A common number shared by two or more points indicates no significant difference* ($p < 0.1$) *for those points.*

$C/k$ was added to estimate the area to infinity ($AUC_\infty$). The renal clearance for aspirin ($CL_r$) was computed by dividing the amount of aspirin accumulated in the urine during the first 0–2-h interval by the area under the plasma curve for the corresponding time interval. Statistical moments (1, 3, 4) were used to compute the mean residence time for aspirin (MRT). An estimate of the residence time in the GI tract (MAT) for each dose was obtained by subtracting 16.2 min from each MRT value; 16.2 min approximates (4) the reported mean residence time for aspirin following intravenous administration and does not reflect experimental data from this report.

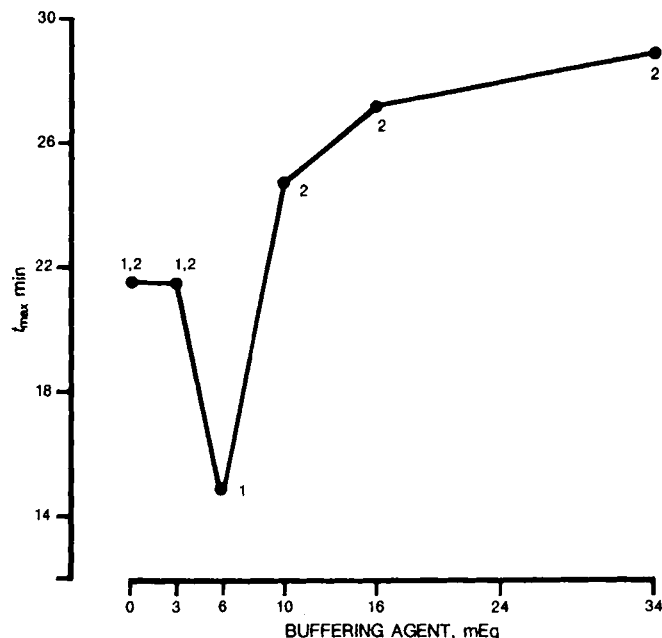


**Figure 2**—*A plot of $t_{max}$ for aspirin as a function of buffer strength. A common number shared by two or more points indicates no significant difference* ($p < 0.1$) *for those points.*

## RESULTS

In this study, the assay method had a lower limit of quantitation of 0.25 μg/mL and a relative coefficient of variation of 5%. Significant differences in the plasma aspirin concentration *versus* time curves, as presented in Table II, were observed for the various amounts of buffer in the dose. Although adjusted means (*i.e.*, least-square means) are employed in the balanced incomplete block design, these plots accurately reflect the plots for individual subjects. Unbuffered doses provided the highest aspirin concentration at the 5-min sample, while those with small amounts (*i.e.*, 3, 6, and 10 mEq) of buffer presented the most rapid rise between 5 and 10 min. With 34 mEq of the buffering agent there is a definite lag time preceding the appearance of aspirin in the plasma, followed by a rapid rise between the 10- and 20-min samples. All curves reached a maximum before 45 min with a subsequent monoexponential decay for which the mean half-lives ranged between 17.0 and 22.5 min across the six treatments.

Estimates of the comparative rate of aspirin absorption among the six different buffer strengths were obtained by referring to the mean $C_{max}$, $t_{max}$, MRT, and MAT values presented in Table III, and plotted in Figs. 1, 2, and 3. Although only some of the values are statistically significant ($p < 0.10$), the doses with 3 and 6 mEq of the buffer appear to provide higher $C_{max}$ and lower $t_{max}$ than unbuffered doses and for those doses with greater amounts of the buffering agent. This same trend is reflected in the statistical moment computations as expressed by the MAT and MRT values. The use of a 0.1 level of significance in computing the least significant differences is justified because the observations at each buffer level are not isolated; rather, they are

**Table II—Plasma Aspirin Concentrations** [a]

| Time, min | Antacid Buffering Agent, mEq | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 3 | 6 | 10 | 16 | 34 |
| Predose | —[b] | —[b] | —[b] | —[b] | —[b] | —[b] |
| 5 | 5.00 | 0.84 | 2.61 | 4.50 | 3.56 | 0.49 |
| 10 | 7.29 | 7.60 | 12.6 | 8.98 | 6.70 | 1.97 |
| 15 | 10.7 | 13.9 | 13.8 | 13.8 | 12.1 | 5.47 |
| 20 | 11.8 | 17.3 | 16.0 | 10.5 | 12.5 | 7.49 |
| 30 | 11.3 | 12.7 | 11.3 | 9.26 | 9.91 | 8.15 |
| 45 | 6.71 | 6.16 | 4.72 | 6.94 | 7.04 | 5.86 |
| 60 | 3.70 | 3.17 | 2.22 | 4.12 | 3.53 | 3.84 |
| 90 | 1.38 | 1.11 | 0.72 | 1.38 | 0.90 | 1.16 |
| 120 | 0.43 | 0.40 | 0.25 | 0.60 | 0.41 | 0.42 |
| 150 | —[b] | —[b] | —[b] | —[b] | —[b] | —[b] |

[a] Adjusted means are least-squares means for the balanced incomplete block design. [b] Below detectable limit of 0.25 μg/mL.

**Table III—Adjusted Means for Plasma Aspirin Parameters** [a]

| Parameters [b] | | | | | | |
|---|---|---|---|---|---|---|
| $AUC_\infty$, μg·min/mL | | | | | | |
| Buffer, mEq | 3 | 0 | 10 | 6 | 16 | 34 |
| Adjusted Mean | 599 | 586 | 578 | 560 | 554 | 420 |
| $C_{max}$, μg/mL | | | | | | |
| Buffer, mEq | 6 | 3 | 16 | 10 | 0 | 34 |
| Adjusted Mean | 17.8 | 17.3 | 16.2 | 15.8 | 13.4 | 8.6 |
| $t_{max}$, min | | | | | | |
| Buffer, mEq | 6 | 3 | 0 | 10 | 16 | 34 |
| Adjusted Mean | 14.8 | 21.5 | 21.5 | 24.8 | 27.3 | 29.0 |
| Urine, pH, 0–2 h | | | | | | |
| Buffer, mEq | 6 | 16 | 0 | 3 | 10 | 34 |
| Adjusted Mean | 5.5 | 5.5 | 5.7 | 5.9 | 6.0 | 7.1 |
| $CL_r$, L/h | | | | | | |
| Buffer, mEq | 6 | 0 | 10 | 3 | 16 | 34 |
| Adjusted Mean | 0.80 | 0.84 | 0.84 | 0.92 | 1.33 | 1.96 |
| 24-h Dose, % [c] | | | | | | |
| Buffer, mEq | 16 | 10 | 3 | 6 | 0 | 34 |
| Adjusted Mean | 60.4 | 63.2 | 64.5 | 66.3 | 70.6 | 71.0 |
| MRT, min | | | | | | |
| Buffer, mEq | 6 | 3 | 16 | 0 | 10 | 34 |
| Adjusted Mean | 32.9 | 36.3 | 38.4 | 40.1 | 42.5 | 45.7 |
| MAT [c], min | | | | | | |
| Buffer, mEq | 6 | 3 | 16 | 0 | 10 | 34 |
| Adjusted Mean | 16.7 | 20.1 | 22.2 | 23.9 | 26.3 | 29.5 |
| $t_{1/2}$, min | | | | | | |
| Buffer, mEq | 3 | 0 | 6 | 16 | 10 | 34 |
| Adjusted Mean | 17.0 | 18.0 | 18.5 | 18.8 | 21.3 | 22.6 |

[a] Least-squares means for balanced incomplete block design; a line under two or more values indicates no significant difference ($p < 0.1$). [b] See text for definition of terms. [c] Sum of aspirin, salicylic acid, and salicyluric acid recovered in urine and corrected for formula weight.

integral members of a sequence of buffer strengths. To require each to meet the 0.05 level of significance, independently, would be an excessively severe test.

Estimation of the extent to which aspirin reaches the circulatory system requires consideration of both the $AUC_\infty$ and the $CL_r$ for aspirin. The significantly lower $AUC_\infty$ for aspirin with 34 mEq of the buffering agent, compared with aspirin with lesser amounts of the buffering agent, as presented in Table I, can partially be attributed to an increased renal clearance as the pH of the urine increased. While the observed increase in renal clearance from 0.84 L/h, with no buffering, to 1.96 L/h, with 34 mEq of the buffering, is significant ($p < 0.10$), the overall maximal increase in systemic aspirin clearance in urine is ~2–3%, if the systemic clearance is ~40 L/h as previously reported for similar subjects (1, 5). Based on these clearance changes one might expect a 2–3% lower $AUC_\infty$ with 34 mEq of the buffering agent, rather than the 28% lower $AUC_\infty$ shown in Table III. These data suggest that adding 34 mEq of the buffering agent to the aspirin dose results in about 25% less aspirin reaching the circulatory system.

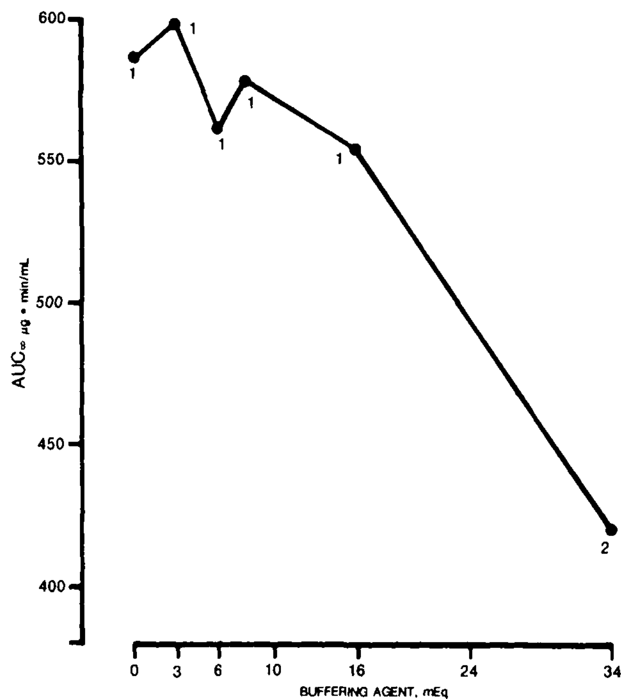


**Figure 3**—*A plot of $AUC_\infty$ for aspirin as a function of buffer strength. A number shared by two or more points indicates no significant difference (p < 0.1) for those points.*

Although less aspirin reaches the circulatory system with the 34 mEq of the buffering agent, the total salicylate (*i.e.*, aspirin, salicylic acid, and salicyluric acid) which accumulates in the urine over 24 h is not decreased. Combining these observations suggests that the absorption of aspirin is not diminished by added buffering agents, but that the presystemic hydrolysis is increased. This is due to a greater portion of aspirin being emptied into and absorbed from the small intestine, where significant esterase activity has been reported to exist (6).

## DISCUSSION

Aspirin was rapidly absorbed from all of the solutions, while the rate and extent of the bioavailability was dependent on buffer strength. Small quantities of the antacid buffering agent were associated with the most rapid rise in plasma aspirin, with mean maximal concentrations of 17.8 and 17.3 μg/mL for 6 and 3 mEq, respectively. This rapid rate of absorption was probably due to the increased solubility of the aspirin, with only a small increase in the pH of gastric contents and, thus, a minimal suppression of gastric absorption. In the absence of buffering the aspirin was only partly dissolved before reaching the stomach, which provided a rapid rate of absorption over the first few minutes only.

With 34 mEq of the antacid buffer, the gastric pH may rise above six and suppress absorption through the gastric mucosa (7, 8). Indeed, absorption would be predominately delayed until the aspirin was emptied into the small intestine, where the buffering effect of the antacid would be diminished and the surface area is much larger. The actual delay in absorption due to the increased gastric pH was only ~5 min, which was probably due to the acceleration of gastric emptying that is generally associated with administration of the sodium citrate–bicarbonate antacid (8).

The greater presystemic hydrolysis observed with the 34 mEq of buffering is consistent with the above hypothesis (*i.e.*, that the antacid can shift the absorption from the stomach to the intestine). Studies in dogs (9) and rats (10) support the supposition that a significant portion of the first-pass hydrolysis of aspirin occurs in the gut wall (6). With the gap between the 16- and 34-mEq strengths of the buffering agent used in the present study, one can only conclude that buffer quantities in excess of 16 mEq are required to significantly alter aspirin absorption kinetics.

Repeated contact with aspirin has been reported (11) to alter the gastric

membrane, allowing hydrogen-ion back-diffusion, which results in irritation, bleeding, and possible ulceration. If this mechanism is correct, then the rapid onset of absorption obtained with unbuffered solutions or solutions with less than 16 mEq of antacid buffering may not reduce the incidence of untoward effects as effectively as those containing a greater quantity of antacid buffering.

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# Determination of Ethoxzolamide in the Iris/Ciliary Body of the Rabbit Eye by High-Performance Liquid Chromatography: Comparison of Tissue Levels Following Intravenous and Topical Administrations


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**Abstract** □ A specific high-performance liquid chromatographic method is described for ethoxzolamide following the extraction of the material from iris/ciliary body eye tissue in rabbits. The steps consist of base extraction and protein and enzyme deactivation, followed by acid treatment, extraction into ethyl acetate, evaporation, and solubilization with a 50% aqueous methanol solution. The samples were chromatographed on a reverse-phase phenyl column with a mobile phase consisting of 50% methanol in 1% acetic acid. The recovery was 74.3% over a 10-fold range of tissue concentrations. The sensitivity was 0.03 μg/mL, and the response was linear over the concentration range (0.03–0.5 μg/mL) used in the study. Intravenous (2.0- and 6.0-mg/kg) and topical (1% suspension) doses of ethoxzolamide were administered to rabbits. Iris/ciliary body tissues were excised 45 min after drug administration. The tissue levels after a dose of 6 mg/kg were statistically greater than the levels obtained after a dose of 2 mg/kg. The smaller intravenous dose represented the lowest dose for which a reduction in intraocular pressure could be measured. An initial transitory drop in intraocular pressure was detected for the topical dose. Iris/ciliary body levels in the treated eye could be detected for the 2-mg/kg iv and topical doses.



**Keyphrases** □ Ethoxzolamide—determination in the iris/ciliary body, rabbits, HPLC, GC □ Dosage forms—intravenous and topical administration of ethoxzolamide, iris/ciliary body of rabbit eyes, HPLC, GC


Ethoxzolamide, a carbonic anhydrase inhibitor used in glaucoma to lower intraocular pressure (IOP), is effective orally but not when administered topically to the eye, presumably due to inadequate drug levels at the active site. In recent studies, the anatomical location of carbonic anhydrase with the anatomical location of aqueous humor formation in the ciliary body have been identified (1, 2). Friedlander and Muther (3) have utilized the high binding affinity of [$^3$H]-acetazolamide for carbonic anhydrase to demonstrate the distribution of carbonic anhydrase in the epithelium of the ciliary body. The greater specificity of ethoxzolamide for the enzyme was shown by displacing [$^3$H]acetazolamide with a high concentration of unlabeled ethoxzolamide.

Although Maren (4) have devised a sensitive method for the determination of carbonic anhydrase and its inhibitors, the assay measures drug activity; therefore, specificity is always in doubt. It is also tedious and time consuming. It was the purpose of this study to develop a high-performance liquid chromatographic (HPLC) procedure for the determination of ethoxzolamide in the iris/ciliary body tissue of rabbit eyes. Data are also presented which compare tissue concentrations after administration by the topical and intravenous routes.

## EXPERIMENTAL SECTION

**Chemicals**—Ethoxzolamide, a gift from a commercial source[1], was used as received. The substances used in the preparation of the various buffers and vehicles were of analytical or USP quality, and included monobasic sodium phosphate[2], dibasic sodium phosphate[3], sodium carbonate[2], hydrochloric acid[2], sodium chloride[4], polysorbate 80[5], and *N,N*-dimethylacetamide[2]. Ethyl acetate[6] was used as the organic phase in the extraction procedure. Acetic acid[7], methanol[8], and distilled deionized water were used to prepare the chromotographic mobile phase. The distilled deionized water was also used to make all aqueous dilutions.

**Apparatus**—The chemicals and tissues were weighed on an electronic[9] or analytical[10] balance. The pH values of the various solutions were measured[11] when necessary.

The HPLC system consisted of a solvent delivery pump[12], a syringe-loading sample injector[13] fitted with a 100-μL loop[14], a reverse-phase phenyl column[15], and a fixed-wavelength UV detector[16] operating at 313 nm. Chromatograms were recorded on a chart recorder[17] operating at 0.5 cm/min. The mobile phase consisting of methanol–1% aqueous acetic acid (50:50, v/v) was deaerated before use; the flow rate was 1.5 mL/min.

[1] The Upjohn Co.
[2] Certified ACS; Fisher Scientific Co., Fair Lawn, N.J.
[3] Baker Analyzed Reagent; J. T. Baker Chemical Co., Phillipsburg, N.J.
[4] USP grade; J. T. Baker Chemical Co.
[5] USP grade; Ruger Chemical Co., Inc., Irvington, N.J.
[6] Analytical Reagent ACS; Mallinckrodt Inc., Paris, Ky.
[7] Certified ACS; Mallinckrodt Inc.
[8] HPLC grade; Burdick & Jackson Laboratories, Inc., Muskegon, Mich.
[9] Model 4100; Cahn Instruments, Paramount, Calif.
[10] Model B6; Mettler Instruments Corp., Hightstown, N.J.
[11] Model 701; Orion Research Corp., Cambridge, Mass.
[12] Model 6000A; Waters Associates, Milford, Mass.
[13] Model 7125; Rheodyne Inc., Cotati, Calif.
[14] Catalog no. 724; Rheodyne Inc.
[15] P/N 27198, S/N μ-Bondapak Phenyl; Waters Associates.
[16] Model 440 absorbance detector; Waters Associates.
[17] Model A5211-1; Omniscribe, Houston Instruments, Austin, Tex.


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