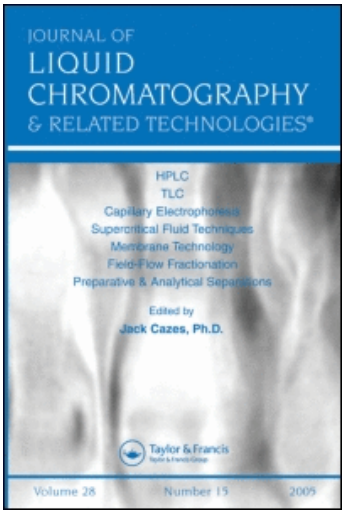


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Separation of Sulfisoxazole, Phenazopyridine, and Their Related Impurities by Micellar Electro-Kinetlc Chromatography

Beverly Nickerson[a]; Stephen Scypinski[a]; Helen Sokoloff[b]; Swroop Sahota[b]
[a] Analytical Research and Development, Hoffmann-La Roche Inc., Nutley, New Jersey [b] Pharmaceutical Quality Control and Quality Assurance, Hoffmann-La Roche Inc., Nutley, New Jersey

To cite this Article Nickerson, Beverly , Scypinski, Stephen , Sokoloff, Helen and Sahota, Swroop(1995) 'Separation of Sulfisoxazole, Phenazopyridine, and Their Related Impurities by Micellar Electro-Kinetlc Chromatography', Journal of Liquid Chromatography & Related Technologies, 18: 18, 3847 — 3875
To link to this Article: DOI: 10.1080/10826079508014629
URL: http://dx.doi.org/10.1080/10826079508014629

JOURNAL OF LIQUID CHROMATOGRAPHY, 18(18&19), 3847-3875 (1995)

SEPARATION OF SULFISOXAZOLE, PHENAZOPYRIDINE, AND THEIR RELATED IMPURITIES BY MICELLAR ELECTRO-KINETIC CHROMATOGRAPHY

BEVERLY NICKERSON[1]*, STEPHEN SCYPINSKI[1], HELEN SOKOLOFF[2], AND SWROOP SAHOTA[2]
[1]*Analytical Research and Development*
[2]*Pharmaceutical Quality Control and Quality Assurance*
Hoffmann-La Roche Inc.
340 Kingsland Street
Nutley, New Jersey 07110-1199

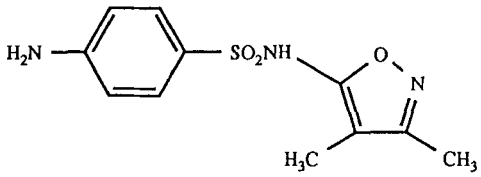
ABSTRACT

A micellar electrokinetic chromatographic method for the analysis of sulfisoxazole, phenazopyridine and their related known impurities in a tablet dosage form has been developed and validated. Current methods employed for this dosage form involve the use of two separate ultraviolet spectrophotometric or titrimetric assay procedures for the determination of the actives. Traditional reversed-phase high performance liquid chromatographic separations of sulfisoxazole and its major degradation products, sulfanilamide and sulfanilic acid, result in the elution of the latter two components at or near the void volume, causing problems with their identification and quantitation. The micellar electrokinetic chromatographic method described here employs sodium dodecyl sulfate as the surfactant and effectively separates sulfisoxazole, phenazopyridine and all but two of their related impurities within thirty minutes. The method was found to be valid with respect to specificity, linearity and limits of detection. System precision, however, was relatively poor. Application of the method to the quantitation of these components in a tablet dosage form is presented.

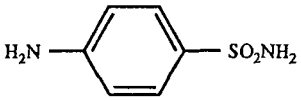
INTRODUCTION

Azo Gantrisin[R] is a tablet dosage form used for the treatment of uncomplicated urinary tract infections. It is composed of the antibacterial compound sulfisoxazole and the urinary analgesic phenazopyridine. Figures 1 and 2 show the structures of sulfisoxazole, phenazopyridine and their related impurities. Current testing methodologies for quantitating sulfisoxazole and phenazopyridine involve the use of separate ultraviolet (UV) spectrophotometric or titrimetric assay methods [1-4]. It was desired to develop a single chromatographic method to simultaneously quantitate these two substances. Efforts to develop a reversed-phase high performance liquid chromatographic (HPLC) method to separate sulfisoxazole, phenazopyridine and their related impurities were unsuccessful. Reversed-phase HPLC separations developed in our laboratory for the separation of sulfisoxazole and its two degradation products, sulfanilamide and sulfanilic acid, resulted in the elution of the latter two components at or near the void volume, causing problems with their identification and quantitation. The use of a basic ion-pairing reagent caused sulfanilamide and sulfanilic acid to be retained; however, other components in the sample matrix exhibited either a lack of retention or excessive retention [5]. The use of alternate separation techniques from reversed-phase HPLC were therefore explored for this dosage form.

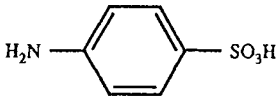
Azo Gantrisin[R], which contains a combination of two active substances, was deemed a good model for testing the utility of micellar electrokinetic chromatography (MEKC), also referred to as micellar electrokinetic capillary chromatography (MECC), for practical pharmaceutical analysis. There are numerous examples in the literature



Sulfisoxazole



Sulfanilamide

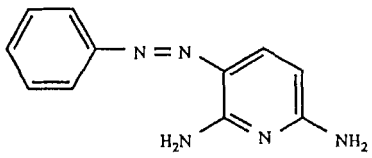


Sulfanilic Acid

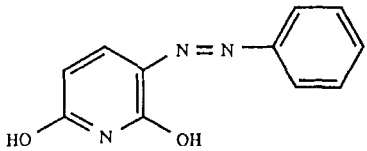
Figure 1

Chemical structures for sulfisoxazole and two of its potential degradation products, sulfanilamide and sulfanilic acid.

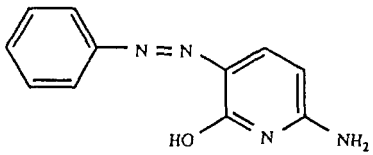
where capillary zone electrophoresis (CZE) [6-10] and MEKC [6, 11-14] have been employed as an alternative or complementary technique to HPLC in the analysis of "small-molecule" pharmaceuticals. MEKC is a variation of the increasingly popular technique of CZE [15-19]. In MEKC, surfactant molecules present at a sufficient concentration to form micelles are used to differentially solubilize analyte molecules based on their degree of hydrophobicity. The charged micelles are subject to electrophoretic effects and therefore migrate at a different rate than the surrounding aqueous phase. These micelles then behave as a moving chromatographic stationary phase and effectively separate molecules which have little or no differences in their electrophoretic mobilities but which do have different hydrophobicities [20-22].



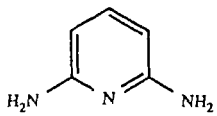
Phenazopyridine



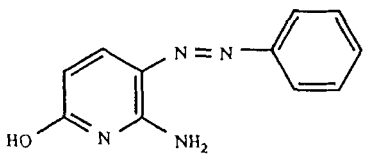
3-(Phenylazo)-2,6-pyridinediol



6-Amino-3-(phenylazo)-2-pyridinol



2,6-Diaminopyridine



6-Amino-5-(phenylazo)-2-pyridinol

Figure 2

Chemical structures for phenazopyridine and four of its possible synthetic by-products and degradation products.

MEKC was evaluated as a possible means of separating and quantitating sulfisoxazole, phenazopyridine and their associated known impurities. Using a phosphate buffer system and sodium dodecyl sulfate as the micelle carrier, it was possible to separate all the analytes of interest except for the geometrically isomeric 3-

and 5-(phenylazo) pyridinols from each other within a run time of thirty minutes. This technique was demonstrated to be feasible for the complete separation of both active components and their related impurities in the tablet dosage form. The method is fairly rapid, simple and has been validated with respect to specificity, precision, linearity and detection limits.

MATERIALS

Sulfisoxazole and phenazopyridine were obtained from Hoffmann-La Roche Inc. (Nutley, NJ). 6-Amino-3-(phenylazo)-2-pyridinol, 6-amino-5-(phenylazo)-2-pyridinol, 2,6-diaminopyridine and 3-(phenylazo)-2,6-pyridinediol were obtained from F. Hoffmann-La Roche AG (Basel, Switzerland). Sulfanilamide was obtained from Eastman Kodak Company (Rochester, NY) and sulfanilic acid was purchased from MC&B (Cincinnati, OH). Sodium phosphate monobasic, sodium phosphate dibasic and sodium dodecyl sulfate were purchased from Fisher Scientific (Fair Lawn, NJ). All reagents and chemicals were used as received. Water was distilled and deionized using a NANOpure analytical grade system (Barnstead/Thermolyne Corporation, Dubuque, IA).

PROCEDURES

Preparation of Buffer

The buffer used to perform micellar electrokinetic chromatography consisted of 5 mM sodium phosphate monobasic, 5 mM sodium phosphate dibasic and 50 mM

sodium dodecyl sulfate. The pH of the buffer was adjusted to 7.0 using sodium hydroxide. This buffer was also used to dissolve and dilute samples.

Preparation of Solutions for Linearity, Limits of Detection and System Precision Studies

A solution containing 1 mg/mL sulfisoxazole and 0.1 mg/mL phenazopyridine was prepared by weighing 500 mg sulfisoxazole and 50 mg phenazopyridine into a 500 mL volumetric flask. Approximately 400 mL of buffer was added and the solution was sonicated for 30 minutes to completely dissolve the active components. The solution was then cooled and diluted to volume with buffer. Dilutions of this stock solution to perform the linearity and sensitivity studies for sulfisoxazole and phenazopyridine were made with buffer to the desired concentration.

Several stock solutions of the impurities were prepared as follows. A sulfisoxazole impurities stock solution containing 0.2 mg/mL each of sulfanilamide and sulfanilic acid was made by dissolving 5 mg of each of these compounds in 25 mL of sulfisoxazole/phenazopyridine stock solution using sonication as needed. A phenazopyridine impurities stock solution containing 0.04 mg/mL each 6-amino-5-(phenylazo)-2-pyridinol, 2,6-diaminopyridine and 3-(phenylazo)-2,6-pyridinediol was made by dissolving 1 mg of each of these standards in 25 mL of sulfisoxazole/phenazopyridine stock solution using sonication as needed. A stock solution containing 0.04 mg/mL 6-amino-3-(phenylazo)-2-pyridinol, another phenazopyridine impurity, was made by dissolving 1 mg of this compound in 25 mL of sulfisoxazole/phenazopyridine stock solution using sonication as needed.

One mL of the sulfisoxazole impurities stock solution, 0.5 mL of the phenazopyridine impurities stock solution and 0.5 mL of the stock solution containing 6-amino-3-(phenylazo)-2-pyridinol were diluted to 10 mL with sulfisoxazole/phenazopyridine stock solution. This resulting solution contained 1 mg/mL sulfisoxazole, 0.1 mg/mL phenazopyridine, 0.02 mg/mL or 2% each of sulfanilamide and sulfanilic acid with respect to sulfisoxazole and 0.002 mg/mL or 2% each of 6-amino-3-(phenylazo)-2-pyridinol, 6-amino-5-(phenylazo)-2-pyridinol, 2,6-diaminopyridine and 3-(phenylazo)-2,6-pyridinediol with respect to phenazopyridine. This solution, containing 1 mg/mL sulfisoxazole, 0.02 mg/mL of the two sulfisoxazole impurities, 0.1 mg/mL phenazopyridine and 0.002 mg/mL of the four phenazopyridine impurities, was used for method development purposes and to test the separation of the components with the MEKC method.

The above solution was prepared without 6-amino-3-(phenylazo)-2-pyridinol and was used to test the precision of the method. Dilutions of this mixture with sulfisoxazole/phenazopyridine stock solution were used to perform the linearity and sensitivity studies for these impurities.

Preparation of Standard Solution for Assay

Fifty mg of sulfisoxazole and 5 mg of phenazopyridine were accurately weighed into a 50-mL volumetric flask. Approximately 40 mL of buffer was added and the solution was sonicated for 30 minutes to completely dissolve the sulfisoxazole and phenazopyridine. The solution was then cooled and diluted to volume with buffer

to yield a standard containing 1 mg/mL sulfisoxazole and 0.1 mg/mL phenazopyridine. This solution was diluted 1:25 with buffer to yield a solution containing 0.04 mg/mL sulfisoxazole and 0.004 mg/mL phenazopyridine for use in the assay of these components.

Preparation of Tablet Sample Solutions

Ten Azo Gantrisin[R] tablets, each containing 500 mg sulfisoxazole and 50 mg phenazopyridine were ground in a mortar to form a composite mixture. A quantity of this composite equivalent to 50 mg sulfisoxazole and 5 mg phenazopyridine was accurately weighed into a 50 mL volumetric flask and approximately 40 mL of buffer was added. The solution was sonicated for 30 minutes to dissolve the sulfisoxazole and phenazopyridine. The solution was then diluted to volume with buffer and filtered through an HV filter (Millipore, Bedford, MA), 0.45 μm, to provide a solution for the determination of the impurities. A 1:25 dilution of this solution was made with buffer to provide a solution for the assay of sulfisoxazole and phenazopyridine.

Micellar Electrokinetic Chromatography

A Model CES I capillary electrophoresis system (Dionex Corporation, Sunnyvale, CA) was used to perform micellar electrokinetic chromatography. The fused-silica capillary was 75 μm x 70 cm and the detection window was formed as described in the CES I manual [23]. Hydrodynamic injections were performed at a height of 50 mm for 10 seconds. Electrophoresis was performed using a 15 kV (34

mA) potential across the electrodes. After each run, the buffer reservoirs were drained and refilled with fresh buffer (6 second rinse time at 7 psi) and the capillary was rinsed with fresh buffer (180 second rinse time at 7 psi). Detection was performed by measurement of the UV absorbance at 240 nm. Data were collected and analyzed using a Beckman PeakPro chromatographic data system (Beckman Instruments, Fullerton, CA).

RESULTS

Although CZE has been gaining popularity as a technique for the separation of small molecules, MEKC rather than CZE was chosen as the separation technique for this work due to the physico-chemical properties of two impurities of phenazopyridine, 6-amino-3-(phenylazo)-2-pyridinol and 6-amino-5-(phenylazo)-2-pyridinol. These two compounds possess the same mass-to-charge ratio and would therefore not be expected to separate by CZE. By using MEKC, it was hoped that the different locations of the hydroxyl groups would cause different degrees of repulsion of these groups with the negatively charged sodium dodecyl sulfate micelles and hence lead to separation. Unfortunately, this did not prove to be the case. The 3- and 5-(phenylazo) pyridinols could not be resolved by MEKC. All other components were successfully resolved. Figure 3 shows the separation obtained for sulfisoxazole, phenazopyridine and their related impurities. The buffer used to obtain this separation had a pH of 7.0. At buffer pH values of 7.5 to 8.6, 6-amino-5-(phenylazo)-2-pyridinol, 6-amino-5-(phenylazo)-2-pyridinol and phenazopyridine coeluted. By lowering the pH of the

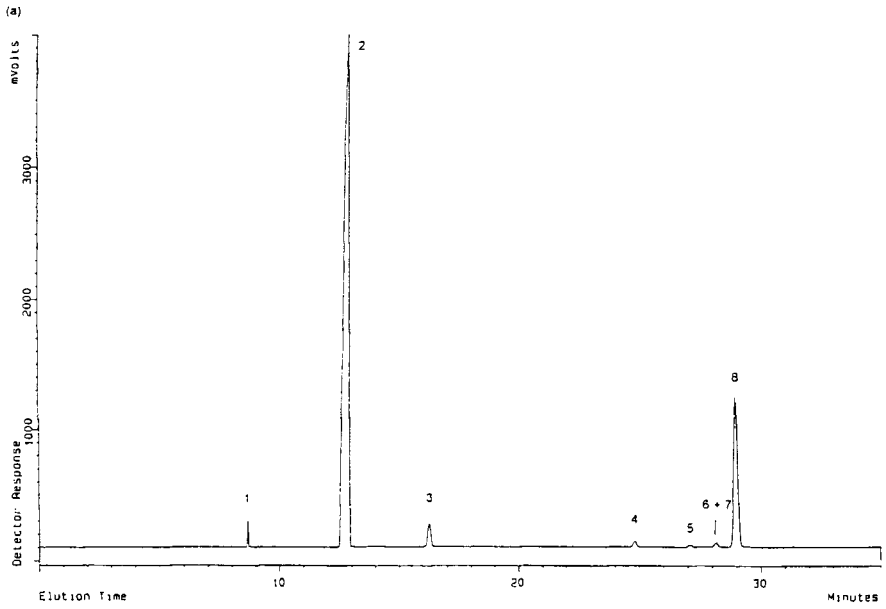


Figure 3

a) Full scale and b) expanded electropherograms showing 2% each of sulfanilamide and sulfanilic acid relative to sulfisoxazole and 2% each of 2,6-diaminopyridine, 3-(phenylazo)-2,6-pyridinediol, 6-amino-5-(phenylazo)-2-pyridinol and 6-amino-3-(phenylazo)-2-pyridinol relative to phenazopyridine. Components: 1 = sulfanilamide; 2 = sulfisoxazole; 3 = sulfanilic acid; 4 = 2,6-diaminopyridine; 5 = 3-(phenylazo)-2,6-pyridinediol; 6 = 6-amino-5-(phenylazo)-2-pyridinol; 7 = 6-amino-3-(phenylazo)-2-pyridinol; and 8 = phenazopyridine.

buffer and increasing the degree of protonation of the amino groups, two amino groups for phenazopyridine and one for the pyridinols, a good separation of phenazopyridine from these two impurities was achieved. The positively charged amine groups interacted with the negatively charged micelles, leading to later elution times. The 3- and 5-(phenylazo) pyridinols, however, eluted as a single well-shaped peak under the

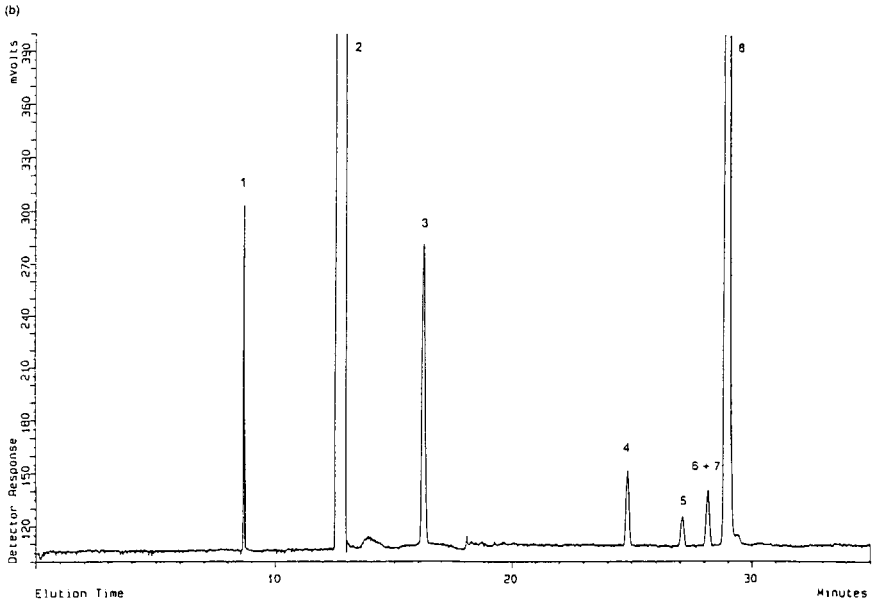


Figure 3 (continued)

conditions of this method. Subsequent work was therefore performed using all the impurities except 6-amino-5-(phenylazo)-2-pyridinol. Once a satisfactory separation had been obtained, the method was validated with respect to several key parameters which provided an indication of its performance. This was done to investigate the possible future utility of this technique for routine analysis.

System Precision

System precision provides a measure of system performance that is independent of the error introduced by sample handling and preparation. Six replicate

TABLE 1

System precision for the retention times (in minutes), t_r, and peak areas (in arbitrary units) for replicate injections of a standard solution containing 0.04 mg/mL sulfisoxazole and 0.004 mg/mL phenazopyridine.

Injection Number	Sulfisoxazole		Phenazopyridine	
	t_r	Peak Area	t_r	Peak Area
1	13.9	3101	36.4	773.4
2	14.0	3650	37.2	898.6
3	14.3	3028	39.0	804.3
4	14.5	3307	40.0	870.7
5	14.6	3023	40.2	754.8
6	14.8	3049	41.4	811.5
Mean	14.4	3193	39.0	818.9
%RSD	2.8	7.8	4.9	6.8

injections of a standard solution containing 0.04 mg/mL sulfisoxazole and 0.004 mg/mL phenazopyridine were made. These are the concentrations used to assay sulfisoxazole and phenazopyridine in Azo Gantrisin^R tablets. The retention times and peak area for sulfisoxazole and phenazopyridine for these six runs are listed in Table 1. The percent relative standard deviation (%RSD) obtained for the retention times was 2.8% and 4.9% for sulfisoxazole and phenazopyridine, respectively. The percent relative standard deviation (%RSD) obtained for the peak areas was found to be 7.8% and 6.8%, for sulfisoxazole and phenazopyridine, respectively.

In this work approximately 6.8 nL of sample are injected onto the capillary. This corresponds to injecting approximately 0.27 ng sulfisoxazole and 0.027 ng phenazopyridine onto the capillary in this system precision study. Considering that the concentrations of both components are in the sub-nanogram range, the system precision obtained is reasonable. It is however, higher than those values typically obtained with HPLC methods. Currently, however, there is no HPLC method for the determination of these two compounds and their impurities.

System precision was also evaluated by performing replicate injections of a standard solution containing 1.0 mg/mL sulfisoxazole, 0.1 mg/mL phenazopyridine, 2% sulfanilamide and sulfanilic acid relative to sulfisoxazole and 2% 6-amino-5-(phenylazo)-2-pyridinol, 3-(phenylazo)-2,6-pyridinediol and 2,6-diaminopyridine relative to phenazopyridine. This mixture was analyzed to provide precision information on the levels of these samples used to quantitate the impurities. A set of ten replicate injections was performed on two different days. These results are summarized in Table 2. It should be noted that the sulfisoxazole peak at this level is non-Gaussian in shape. This is the reason that sulfisoxazole is quantitated at a lower concentration, 0.04 mg/mL. As shown in Table 2, the percent relative standard deviation for the peak areas ranged from 5.8% to 14.8% for day 1 and from 4.5% to 13.4% for day 2. Although values less than 2% RSD for peak areas have been obtained with CZE and MEKC methods, some groups [24-27] have reported higher values which are comparable to those obtained in this work. The %RSD values for peak retention time ranged from 0.77% to 2.5% on day 1 and from 1.2% to 2.6% on

TABLE 2

System precision for retention times (in minutes), t_r, and peak areas (in arbitrary units) for a solution containing the listed components as measured on two different days. The mean values and percent relative standard deviations (%RSD) are calculated for six runs.

Component	Mean t_r (%RSD)		Mean Peak Area (%RSD)	
	Day 1	Day 2	Day 1	Day 2
Sulfanilamide	8.7 (0.77)	9.6 (1.2)	750 (8.0)	750 (5.2)
Sulfisoxazole	13.0 (1.2)	14.6 (1.4)	67000 (5.8)	69600 (4.5)
Sulfanilic Acid	16.4 (1.5)	19.0 (1.6)	1950 (7.2)	2100 (5.7)
2,6-Diamino-pyridine	25.1 (2.4)	31.6 (2.6)	480 (6.9)	540 (8.4)
3-(Phenylazo)-2,6-pyridinediol	27.5 (2.3)	34.9 (2.5)	210 (14.8)	240 (13.4)
6-Amino-5-(phenylazo)-2-pyridinol	28.5 (2.5)	36.5 (2.5)	390 (8.0)	460 (8.9)
Phenazopyridine	29.3 (2.5)	37.8 (2.6)	15000 (6.0)	17000 (5.7)

day 2. In general, the %RSD increased as the retention time increased. This variation in retention times might cause a problem in peak identification for the later eluting peaks. In this work the capillary was rinsed with buffer between runs and the capillary was stored overnight filled with water. It may be possible that a different conditioning regime of the capillary may improve the reproducibility of the retention times. Lauer

and McManigill [28] showed that storing the capillary overnight filled with potassium hydroxide gave a standard deviation of less than 1% on a given day for the migration times of 10 runs and less than 4% from day-to-day for a nonmicellar capillary electrophoresis method. In addition, for an MEKC method for theophylline, Linhares and Kissinger [25] showed that capillary washes with sodium hydroxide gave retention times with 1.3% RSD for 31 runs, and that without these rinses between runs the retention times would increase with time. Different capillary conditioning regimes, however, were not explored in our work.

The percent relative standard deviation for peak area ranged from 5.8% to 14.8% for day 1 and from 4.5% to 13.4% for day 2. Again, these values are high compared to HPLC methods. In general, the percent relative standard deviation increased as the peak area decreased. This trend was also observed in the linearity study, where the impurities were injected in triplicate at concentrations of 0.025% to 2.0% of sulfisoxazole and phenazopyridine. A possible source of error is difficulty in integrating the smaller peaks due to the presence of baseline noise. Since the Dionex CES instrument uses uncapped Eppendorf centrifuge tubes to hold the sample, a second possible source of error is sample evaporation and concentration during the experiment, leading to injection of increasing quantities of solutes. On day 1 of the system precision study, the 10 system precision samples were analyzed immediately after preparation. On day 2, the 10 system precision samples were loaded onto the sample carousel after 22 other samples used for the linearity study. The run time was

30 minutes per sample with an additional 4 or 5 minutes to perform rinses between runs. The 10 system precision samples remained in the sample carousel for over 11 hours before analysis of the first of these samples began. As can be seen in Table 2, the mean peak areas for each component on day 2 is larger than on day 1, possibly due to sample evaporation and concentration. This phenomena may possibly effect the %RSD as well.

Linearity

The linearity of the method with respect to the response of sulfisoxazole, phenazopyridine and their impurities was evaluated. The observed linear ranges for these compounds and the correlation coefficient for the linear least-squares fit lines are listed in Table 3. Measurement of peak area for sulfisoxazole was found to be linear from 0.32 μg/mL to 1000 μg/mL with a correlation coefficient of 1.000. For a 6.8 nL injection volume this range corresponds to 2.2 pg to 6.8 ng injected. In addition, this range represents 1% to 2500% of the expected working concentration for the quantitation of sulfisoxazole. The peak shape for sulfisoxazole at concentrations greater than 40 μg/mL was non-Gaussian due to capillary overloading as would be expected. The phenazopyridine peak area was found to be linear from 0.16 μg/mL to 100 μg/mL with a correlation coefficient of 0.9996. This range corresponds to 1.1 pg to 0.68 ng injected and represents 4% to 2500% of the expected working concentration for the determination of phenazopyridine. These peaks were Gaussian in shape. Concentrations greater than 100 μg/mL phenazopyridine were not examined.

TABLE 3

Linear ranges for the components of interest. The mass linear ranges are calculated based on a 6.8 nL injection volume. The correlation coefficient is for the linear least-squares fit line of the data.

Component	Linear Range		Correlation Coefficient (R)
	Concentration (μγ/mL)	Mass (pg)	
Sulfisoxazole	0.32 - 1000	2.2 - 6800	1.000
Phenazopyridine	0.16 - 100	1.1 - 680	0.9996
Sulfanilamide	0.5 - 20	3.4 - 140	0.9997
Sulfanilic Acid	0.5 - 20	3.4 - 140	0.9997
2,6-Diaminopyridine	0.1 - 2	0.68 - 13.6	0.9995
3-(Phenylazo)-2,6-pyridinediol	0.25 - 2	1.7 - 13.6	0.9995
6-Amino-5-(phenylazo)-2-pyridinol	0.25 - 2	1.7 - 13.6	0.9952

The responses for sulfanilamide and sulfanilic acid were found to be linear from 0.25 μg/mL to 20 μg/mL (3.4 pg to 0.14 ng injected) each with a correlation coefficient value of 0.9998. The 2,6-diaminopyridine response was found to linear from 0.1 μg/mL to 2 μg/mL (0.68 pg to 13.6 pg injected) with a correlation coefficient of 0.9995. The responses for 3-(phenylazo)-2,6-pyridinediol and 6-amino-5-(phenylazo)-2-pyridinol were found to be linear from 0.25 μg/mL to 2 μg/mL (1.7 pg to 13.6 pg) with correlation coefficient values of 0.9995 and 0.9952, respectively. Higher concentrations for the impurities were not examined.

TABLE 4

Limits of detection for the components of interest. The mass limits of detection are calculation based on a 6.8 nL injection volume.

Component	Limits of Detection	
	Concentration (μg/mL)	Mass (pg)
Sulfisoxazole	0.32	2.2
Phenazopyridine	0.16	1.1
Sulfanilamide	0.25	1.7
Sulfanilic Acid	0.25	1.7
2,6-Diaminopyridine	0.10	0.68
3-(Phenylazo)-2,6-pyridinediol	0.25	1.7
6-Amino-5-(phenylazo)-2-pyridinol	0.10	0.68

As demonstrated, the MEKC method provides excellent linearity for the active ingredients, sulfisoxazole and phenazopyridine, as well as for their impurities.

Limits of Detection

The limits of detection for sulfisoxazole, phenazopyridine and their related impurities are listed in Table 4. The limit of detection is defined as the concentration yielding a signal-to-noise ratio of approximately 2. The limit of detection for sulfisoxazole and phenazopyridine was found to be approximately 0.32 μg/mL and

0.16 μg/mL, respectively. These limits represent 0.8% of the 0.04 mg/mL sulfisoxazole and 4.0% of the 0.004 mg/mL phenazopyridine injected in this work for the assay of these components in tablets. For the 6.8 nL injection volume used in this work, these detection limits represent 2.2 pg sulfisoxazole and 1.1 pg phenazopyridine injected onto the capillary. Electropherograms for sulfisoxazole and phenazopyridine at their limits of detection are shown in Figure 4.

The limit of detection for sulfanilamide and sulfanilic acid is 0.25 μg/mL (1.7 pg injected) or 0.025% with respect to the 1.0 mg/mL sulfisoxazole concentration used in this work to quantitate impurities. The limit of detection for 2,6-diaminopyridine and 6-amino-5-(phenylazo)-2-pyridinol is 0.10 mg/mL (0.68 pg) and for 3-(phenylazo)-2,6-pyridinediol it is 0.25 μg/mL (1.7 pg injected). These concentrations correspond to 0.1% and 0.25% of the 0.1 mg/mL phenazopyridine concentration used to quantitate impurities.

The limits of detection of the MEKC method for sulfisoxazole and phenazopyridine, as well as for their impurities, are acceptable for the application presented here.

Assay and Determination of Impurities in Tablets

Two samples of a tablet composite (sample taken from 10 ground tablets) were assayed for sulfisoxazole and phenazopyridine using standards containing 0.04 mg/mL sulfisoxazole and 0.004 mg/mL phenazopyridine and diluting the sample to this concentration based upon label claim. The results of this analysis are listed in Table 5.

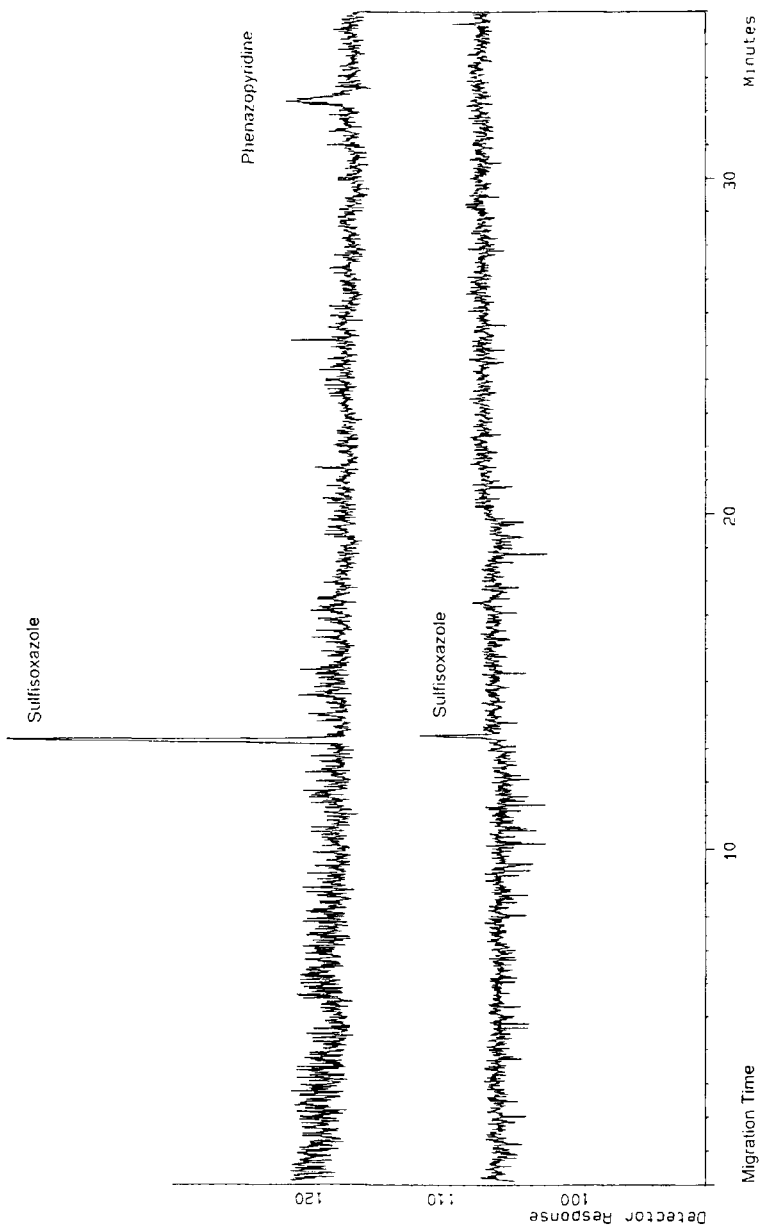


Figure 4

Electropherograms of sulfisoxazole (1.6 μg/mL and 0.32 μg/mL) and phenazopyridine (0.16 μg/mL) at their limits of detection.

TABLE 5

Assay of composite samples of Azo Gantrisin[R] tablets containing sulfisoxazole (500 mg) and phenazopyridine (50 mg).

Injection Number	Sample 1		Sample 2	
	Sulfisoxazole (mg)	Phenazopyridine (mg)	Sulfisoxazole (mg)	Phenazopyridine (mg)
1	518.7	55.35	468.8	53.90
2	447.5	49.95	477.8	56.87
3	481.0	50.93	466.8	47.90
Mean	482.4	52.08	471.1	52.89
% RSD	7.4	5.5	1.2	8.6
% of claim	96.5	104.2	94.2	105.8

Sulfisoxazole was found at 96.5% and 94.2% of claim, while phenazopyridine was found at 104.2% and 105.8% of claim. Figure 5 shows electropherograms of a blank, a standard and one injection for each sample preparation.

Analysis of a composite sample of this lot of tablets for sulfisoxazole and phenazopyridine by UV spectrophotometric methods found 104.4% sulfisoxazole and 103.8% phenazopyridine. The Uniformity of Dosage Units testing (assay of ten individual tablets) by UV spectrophotometric detection found from 92.6% to 104.8% (3.7% RSD) sulfisoxazole and from 105.4% to 107.4% (0.7% RSD) phenazopyridine.

A summary of the assay results for these tablets by MEKC and UV spectrophotometric methods is shown in Table 6. There is good agreement between

Figure 5

Electropherograms of (a) standard (0.04 mg/mL sulfisoxazole, 0.004 mg/mL phenazopyridine), (b) sample 1 and (c) sample 2 used for the composite assay of Azo Gantrisin® tablets.

TABLE 6

Assay results for Azo Gantrisin[R] tablets using MEKC and UV spectrophotometry. UV spectrophotometry results are from an assay of a composite and from the uniformity of dosage units (UDU) which involves assaying 10 individual tablets.

Method of Analysis	% Label Claim	
	Sulfisoxazole	Phenazopyridine
MEKC	95.4	105.0
UV - Assay	104.4	103.8
UV - UDU	92.6 - 104.8 (3.7%RSD)	105.4 - 107.4 (0.7%RSD)

the determinations of sulfisoxazole and phenazopyridine obtained by MEKC and by the UV spectrophotometric methods.

Composite tablet sample preparations were also prepared at a concentration of 1.0 mg/mL sulfisoxazole and 0.1 mg/mL phenazopyridine to detect the presence of any of the known impurities in the samples. A solution containing 0.04% (0.40 μg/mL) each sulfanilamide and sulfisoxazole was also analyzed and used to quantitate these two impurities in the tablet composite samples. Samples 1 and 2 were found to contain 0.06% and 0.05% sulfanilamide and 0.03% and 0.04% sulfanilic acid, respectively. None of the known impurities of phenazopyridine (2,6-diaminopyridine, 3-(phenylazo)-2,6-pyridinediol or 6-amino-3-(phenylazo)-2-pyridinol/6-amino-5-(phenylazo)-2-pyridinol) were detected. Analysis of this lot of tablets by TLC found 0.1% sulfanilamide and no sulfanilic acid. These results are summarized in Table 7.

TABLE 7

Impurity results for Azo Gantrisin[R] tablets using MEKC and TLC. 2,6-diaminopyridine, 3-(phenylazo)2,6-pyridinediol and 6-amino-3-(phenylazo)-2-pyridinol/6-amino-5-(phenylazo)-2-pyridinol were not detected.

	MEKC		TLC
	Sample 1	Sample 2	
Sulfanilamide	0.06%	0.05%	0.1%
Sulfanilic Acid	0.03%	0.04%	None Detected

There is general agreement between the results obtained for the impurities by MEKC and the TLC method. Due to the lower limits of detection for MEKC than TLC, sulfanilic acid was detected by MEKC and not TLC.

DISCUSSION

An MEKC method was evaluated for the analysis of sulfisoxazole, phenazopyridine and their related known impurities in a tablet dosage form. Two impurities of phenazopyridine, 6-amino-5-(phenylazo)-2-pyridinediol and 6-amino-3-(phenylazo)-2-pyridinediol, were not resolved by this method. System precision, limit of detection and linearity studies were therefore performed with sulfisoxazole, phenazopyridine and all their impurities except 6-amino-3-(phenylazo)-2-pyridinediol.

In this work samples of a tablet composite were analyzed at a relatively high concentration, 1.0 mg/mL sulfisoxazole and 0.1 mg/mL phenazopyridine, to allow

optimal detection of impurities. Higher concentrations could not be used due to the solubility limitations of sulfisoxazole. Results obtained for the impurities by MEKC and TLC were comparable. The limits of detection for both sulfanilamide and sulfanilic acid were found to be 0.32 μg/mL (0.03% of the sulfisoxazole concentration) or 2.2 pg injected onto the capillary. The limits of detection for the other impurities were determined to be 0.10 μγ/mL (0.1% of the phenazopyridine concentration; 0.68 pg injected) for 2,6-diaminopyridine and 6-amino-5-(phenylazo)-2-pyridinol, and 0.25 μg/mL (0.25% of the phenazopyridine concentration; 1.7 pg injected) for 3-(phenylazo)-2,6-pyridinediol. These detection limits are acceptable and represent an improvement to the limits of detection obtainable by TLC. The TLC method currently used to quantitate sulfanilic acid and sulfanilamide has an approximate detection limit of 0.05% of a 10 mg/mL standard solution or 0.05 μg applied to the TLC plate.

Lower concentrations of sulfisoxazole and phenazopyridine, 0.04 mg/mL and 0.004 mg/mL, respectively, were used to assay these components in samples of a tablet composite due to the non-Gaussian peak shape of sulfisoxazole at higher concentrations. The samples of tablet composite were found to contain 96.5% and 94.2% of claim for sulfisoxazole and 104.2% and 105.8% of claim for phenazopyridine. Sulfanilamide and sulfanilic acid were also found in these samples. Results obtained for the quantitation of sulfisoxazole and phenazopyridine by MEKC and the UV spectrophotometric methods were comparable. Since this work was undertaken to assess the feasibility of the use of MEKC to analyze a pharmaceutical dosage form, extensive method validation of drug recovery from the dosage form was

not undertaken. If this method were to be pursued in the future for routine use in testing this dosage form, then more extensive validation of drug recovery would need to be performed in order to meet regulatory requirements.

Current testing methodologies for quantitating sulfisoxazole and phenazopyridine involve the use of separate ultraviolet (UV) spectrophotometric or titrimetric assay methods with the use of TLC to monitor the impurities [1-4]. At least three techniques are therefore required to determine sulfisoxazole, phenazopyridine and their known impurities. The micellar electrokinetic chromatographic method described in this paper is a single method which can quantitate both sulfisoxazole and phenazopyridine, as well as their potential known impurities. The MEKC method, however, does have several disadvantages. Two of the phenazopyridine impurities, 6-amino-5-(phenylazo)-2-pyridinol and 6-amino-5-(phenylazo)-2-pyridinol, coelute as a single peak. In addition, the system precision as measured in terms of % RSD for the peak area is poor compared to what is typically obtained for HPLC methods.

CONCLUSIONS

An MEKC method was developed for the analysis of two active ingredients, sulfisoxazole and phenazopyridine, and their known impurities in a tablet dosage form. Two of the known impurities of phenazopyridine were not resolved from each other with this method. There is, however, no method currently available which resolves these two impurities of phenazopyridine. A disadvantage of the method is that samples

need to be analyzed twice, once for the known impurities and a second time for the main components, sulfisoxazole and phenazopyridine. The sample for the analysis of the main components is prepared by diluting the sample used for analysis of the known impurities. Another disadvantage of the method is that the retention times for the peaks tend to increase with time. This trend could pose a problem in identification of closely eluting peaks if numerous samples are run. In addition, the reproducibility of the peak areas is less than that typically obtained with HPLC methods. There is, however, no HPLC method currently available for the analysis of sulfisoxazole, phenazopyridine and their known impurities.

Despite the disadvantages, MEKC offers a chromatographic alternative to the separate, non-selective UV spectrophotometric and titrimetric assays currently employed for sulfisoxazole and phenazopyridine. In addition to the time savings afforded in the determination of the actives, the impurities can be quantitated without the use of a second method such as TLC. The method also offers additional convenience with the use of a commercial instrument which performs sample injections and sample runs automatically.

REFERENCES

1. "Phenazopyridine Hydrochloride," in The United States Pharmacopeia, 23rd ed., United States Pharmacopeial Convention, Inc., Rockville, MD 1994, 1197.

2. "Sulfisoxazole," in The United States Pharmacopeia, 23rd ed., United States Pharmacopeial Convention, Inc., Rockville, MD 1994, 1468.

3. K. W. Blessel, B. C. Rudy, B. Z. Senkowski, "Phenazopyridine Hydrochloride," in Analytical Profiles of Drug Substances, v. 3, K. Florey, Ed., Academic Press, New York 1974, 465-482.

4. B. C. Rudy and B. Z. Senkowski, "Sulfisoxazole," in Analytical Profiles of Drug Substances, v. 2, K. Florey, Ed., Academic Press, New York 1973, 487-506.

5. Tom Perilli, Hoffmann-La Roche Inc., personal communication, 1993.

6. K.D. Altria, N.W. Smith, J. Chromatogr., 538: 506-509 (1991).

7. K.D. Altria, J. Chromatogr., 634: 323-328 (1993).

8. R. Hupoalahti, J. Sunell, J. Chromatogr., 636: 125-132 (1993).

9. M. Korman, J. Vindevogel, P. Sandra, J. Chromatogr., 645: 366-370 (1993).

10. K.D. Altria, Y. L. Chanter, J. Chromatogr. A, 652: 459-463 (1993).

11. Q. X. Dang, L. X. Yan, Z. P. Sun, K. K. Ling, J. Chromatogr., 630: 363-370 (1993).

12. C. Bjergegaard, S. Michaelsen, K. Mortensen, H. Sorensen, J. Chromatogr., 652: 477-485 (1993).

13. H. Nishi, S. Terabe, J. Pharm. Biomed. Anal., 11: 1277-1287 (1993).

14. P. Sun, G. J. Mariano, G. Barker, R. A. Hartwick, Anal. Lett., 27: 927-938 (1994).

15. J. W. Jorgenson, K. D. Lukacs, Science, 222: 266-272 (1983).

16. M. J. Gordon, X. Huang, S. L. Pentoney, Jr., R. N. Zare, Science, 242: 224-228 (1988).

17. A. G. Ewing, R. A. Wallingford, T. M. Olefirowicz, Anal. Chem., 61: 292A-303A (1989).

18. P. D. Grossman, J. C. Colburn, Capillary Electrophoresis Theory & Practice, Academic Press, Inc., New York, 1992.

19. R. Weinberger, Practical Capillary Electrophoresis, Academic Press, Inc., New York, 1993.

20. S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya, T. Ando, Anal. Chem., 56: 113-116 (1984).

21. S. Terabe, K. Otsuka, T. Ando, Anal. Chem., 57: 834-841 (1985).

22. S. Terabe, Y. Miyashita, O. Shibata, E. R. Barnhardt, L. R. Alexander, D. G. Patterson, B. L. Karger, K. Hosoya, N. Tanaka, J. Chromatogr., 516, 23-31 (1990).

23. Dionex Capillary Electrophoresis System I Operator's Manual, Dionex Corporation, Document No. 034196, Revision 01, May 1990.

24. B.R. Thomas, S. Ghodbane, J. Liq. Chromatogr., 16, 1982-2006 (1993).

25. M.C. Linhares, P.T. Kissinger, J. Chromatogr., 615, 327-333 (1993).

26. N. Nguyen, R. Siegler, W. Vincek, "A Capillary Electrophoresis (CE) Method for the Analysis of SK&F 108566 in Pharmaceutical Dosage Forms," presented at the Fourth Annual Frederick Conference on Capillary Electrophoresis, October 19-20, 1993.

27. H.K. Jones, M. Laskovics, "Chiral Electrophoresis versus HPLC for the Screening of Chiral Precursors of a Serotonin 5-HT^2 Antagonist," presented at the Fourth Annual Frederick Conference on Capillary Electrophoresis, October 19-20, 1993.

28. H. H. Lauer, D. McManigill, Anal. Chem., 58, 168-170 (1986).

Received: July 9, 1995
Accepted: August 2, 1995