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Report

Quantitative Fourier Transform-Infrared/Attenuated Total Reflectance (FT-IR/ATR) Analysis of Trimethoprim and Sulfamethoxazole in a Pharmaceutical Formulation Using Partial Least Squares

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An alternative procedure for the simultaneous determination of trimethoprim and sulfamethoxazole in an intravenous pharmaceutical formulation is presented. Infrared spectra of 14 calibration and 6 validation samples were collected using Fourier transform-infrared/attenuated total reflectance (FT-IR/ATR). Partial least-squares (PLS) analysis of the spectral data yielded an average relative error of prediction of 0.69% for trimethoprim and 0.38% for sulfamethoxazole. Analysis of a commercial formulation gave concentration values for sulfamethoxazole and trimethoprim in close agreement with those obtained by a modification of the high-performance liquid chromatography (HPLC) assay listed in the *United States Pharmacopeia* (USP).

KEY WORDS: Fourier transform-infrared/attenuated total reflectance (FT-IR/ATR); multicomponent analysis: trimethoprim; sulfamethoxazole; partial least squares.

INTRODUCTION

Trimethoprim and sulfamethoxazole occur in combination in a number of dosage forms, including tablets, a suspension, and a concentrate for injection. The combination is indicated for a variety of infections. The USP monograph (1,2) for the concentrate for injection lists a high-performance liquid chromatographic (HPLC) method as the official assay procedure for quality control. The goal of this work was to demonstrate an alternative FT-IR/ATR technique with the ability to determine simultaneously the above-mentioned actives in a commercial intravenous formulation without any preliminary sample treatment, with an acceptable accuracy and precision, and with time requirements similar to those for the HPLC methodology.

Two developments in the field of quantitative infrared spectroscopy have made this study possible. The first was the design of liquid cells employing the principles of internal reflection spectroscopy (3). A beam of radiation entering a suitable crystal material immersed in a sample will undergo internal reflection as long as the angle of incidence of the beam is greater than the critical angle of the crystal material. However, on each reflection the beam penetrates slightly into the surrounding sample, where absorption of radiation at characteristic wavelengths for the sample takes place. For example, the depth of penetration at an angle of incidence of 45°, with zinc selenide as the crystal material, is about 1.5 μm (4). Thus by controlling the length of the internal reflection element, a short and reproducible pathlength can be obtained which allows the strong absorption characteristics of water in the mid-infrared region to be overcome. The measured attenuated radiation yields an infrared spectrum similar to one obtained in a normal transmission mode.

The second development is the use of multivariate statistical methods coupled with computerized spectrometers for quantitative spectral analyses. A review of the partial least-squares (PLS) regression method is presented by Martens (5), while Geladi and Kowalski provide a tutorial (6) and examples (7) of the method with simulated data. Haaland and Thomas (8–10) have compared the PLS method to other quantitative multivariate calibration methods. The advantages of such methods include the ability to perform quantitative analysis on multicomponent mixtures with overlapping bands, to calibrate using mixtures allowing the modeling of interactions between components, and to use combinations of multiple numbers of wavelengths in the calibration. In the present study, not only do trimethoprim and sulfamethoxazole have overlapping spectra, but their spectral contributions are buried under the strong absorptions of the cosolvent vehicle.

MATERIALS AND METHODS

Instrumentation

Spectra were obtained from a Nicolet Model 5DXB spectrometer. The spectrometer is equipped with a deuter-

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ated triglycine sulfate (DTGS) detector and operated under a dry air purge. The spectrometer is interfaced to the Nicolet 1280 processor, which runs the PLS software package (Version 3.01) purchased from Nicolet. Spectra were the result of 300 coadded scans, collected over about 5 min, at 4-cm^{-1} resolution.

The Circle Cell attachment was purchased from Spectra Tech, Inc., Stamford, Conn. The accessory is mounted in the sample compartment of the FT-IR and contains a zinc selenide crystal as the internal reflection element. The HPLC system consisted of a Shimadzu Model LC-6A pump with a SPD-6A variable wavelength detector and C-R3A integrator interfaced with a dual floppy drive for storing and retrieving of chromatographic data. An Alltech C-18 Versapack (25 × 0.46 cm, 10 μm) column with a flow rate of 2.0 ml/min and a 20-μl injection loop were employed. The mobile phase consisted of 20% acetonitrile and 0.1% triethylamine in distilled deionized water adjusted to pH 5.9 with HCl. The variable wavelength detector was set at 275 nm.

Reagents

The following reagents were used as received in the preparation of calibration and validation samples: trimethoprim, sulfamethoxazole, diethanolamine, and benzyl alcohol (Sigma); propylene glycol (Fisher Scientific); sodium metabisulfite (Aldrich); and absolute ethyl alcohol (Aaper). NaOH and HCl solutions (Fisher Scientific) were used for pH adjustments. Septra I.V. Infusion (Lot No. 7W1339; Burroughs Wellcome, Research Triangle Park, N.C.) was the commercial product tested. The mobile phase was prepared using HPLC-grade acetonitrile (EM Science), triethylamine (Fisher Scientific), and distilled deionized water.

Sample Preparation

The 20 samples for calibration and validation were prepared to span a concentration range for both trimethoprim and sulfamethoxazole of ±25% of the labeled concentration of the commercial product. Appropriate amounts of trimethoprim and sulfamethoxazole were weighed into tared 10-ml volumetric flasks and appropriate volumes of stock solutions containing the vehicle additives were employed to afford solution and proper concentration of vehicle contents. The solutions were adjusted to volume and a pH of 10.00 ± 0.18 with NaOH and distilled deionized water. For all samples the final solution consisted of a vehicle containing 40% (w/v) propylene glycol, 10% (w/v) ethyl alcohol, 0.3% (w/v) diethanolamine, with 1.0% (w/v) benzyl alcohol and 0.1% (w/v) sodium metabisulfite added as preservatives in distilled deionized water.

For analysis, the solutions were poured into the Circle Cell to cover completely the zinc selenide crystal (5 ml) and 5 min of purge was allowed before collecting the spectra, giving a total analysis time of approximately 10 min per sample. The ratio of the sample's single-beam spectrum over the spectrum of the empty Circle Cell was computed and stored on a floppy disk.

HPLC Samples

The Septra I.V. Infusion and standard preparation were prepared for injection and sample concentration values calculated according to the USP official monograph (2) for sulfamethoxazole and trimethoprim concentrate for injection. Retention times were 3.85 and 6.70 min, respectively, for sulfamethoxazole and trimethoprim, with baseline absorbance values being reached in approximately 9 min. Each peak area was calculated automatically by the integrator.

RESULTS AND DISCUSSION

Spectra of Samples

Figure 1 shows a spectrum of a sample used in the calibration procedure. Also shown is the spectrum of a sample that contains neither trimethoprim nor sulfamethoxazole. The similarity of these two spectra indicates that the major absorptions are due to the nonactive components of the formulation. Using the interactive subtraction technique resident on the Nicolet software, the spectral contributions of the nonactive components can be removed. The result of a 1:1 subtraction of these two spectra is shown in Fig. 2, which qualitatively shows the spectral contributions of sulfamethoxazole and trimethoprim. Due to the small absorbances of the actives compared to other components and spectral overlap of all components, a single peak height or peak area analysis for each component was impossible.

Calibration Procedure

After appropriate concentration information for the calibration and validation samples is entered, the data are mean centered and autoscaled (6,8). This gives equal influence to each component and frequency in the analysis. After this data pretreatment, variance and correlation plots can be generated which aid in the selection of spectral regions for use in the calibration procedure. A variance plot of wavenumbers versus absorbance units, as illustrated in Fig. 3, is employed to determine the spectral regions in which the magnitudes of the changes in absorption intensities are greatest among the reference spectra. A correlation plot indicates which spectral areas are most highly correlated with the concentration

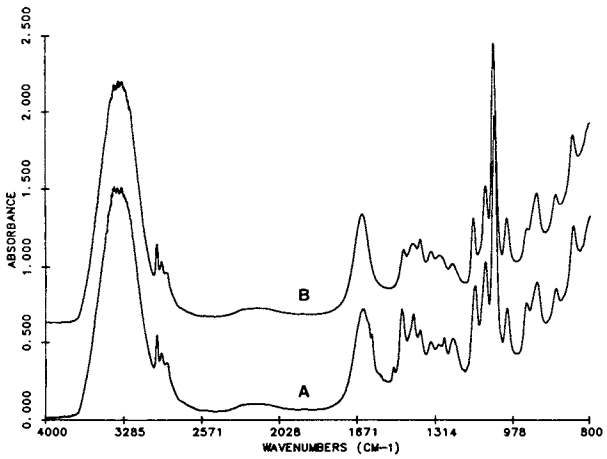

Fig. 1. FT-IR/ATR spectrum (A) of calibration sample containing 16.02 mg/ml of trimethoprim and 80.63 mg/ml of sulfamethoxazole. Spectrum B is an identical formulation except without trimethoprim and sulfamethoxazole.

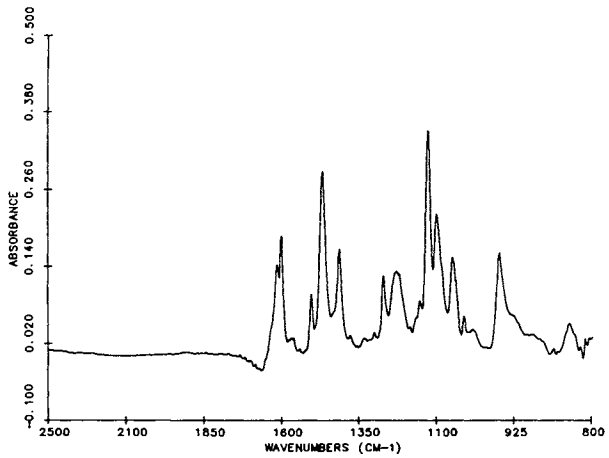

Fig. 2. Subtraction result (1:1) of calibration sample with trimethoprim and sulfamethoxazole minus the spectrum of formulation without actives.

values. Ideal regions for analysis are those with large variances and high correlations with concentration. Regions or groups of regions selected for the calibration are modeled by selecting an optimal number of factors (loading vectors) (8) to represent the complexity of the system without overfitting the concentration data. Nicolet's version of PLS software uses an error analysis of the validation samples to select the number of factors for each component. Table I contains the pertinent information for the calibration model used in this analysis.

Calibration and Validation Results

Figure 4 is a plot of actual versus calculated concentrations for the calibration samples for both trimethoprim and sulfamethoxazole, with each having a correlation coefficient of 0.999. Table II shows, for each validation sample, the real

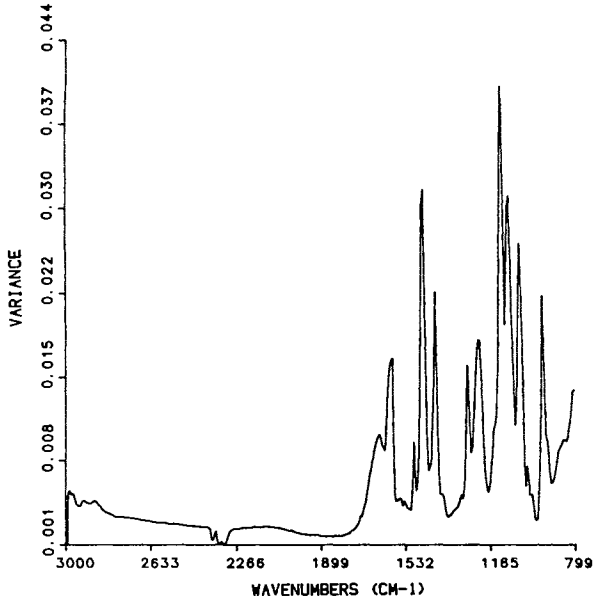

Fig. 3. Variance plot of calibration samples from 3000 to 800 cm^{-1}. Variance is expressed as change in absorbance units.

Table I. Calibration Information for the Model Used in Analysis of Validation and Commercial Samples

Parameter	Trimethoprim	Sulfamethoxazole
Regions[a] (cm^{-1})	1510–1390	1510–1390
	1270–990	1270–990
	960–900	960–900
Number of factors	7	5
% concentration explained[b]	98.31	98.13

[a] Used only absorbance values ≤1.5 in these regions.
[b] Cumulative effect of factors in describing variance for the concentration set of each component.

concentration, the predicted values measured by the instrument, and the relative error for each prediction. The results for both trimethoprim and sulfamethoxazole were excellent, with average relative errors of 0.69 and 0.38%, respectively.

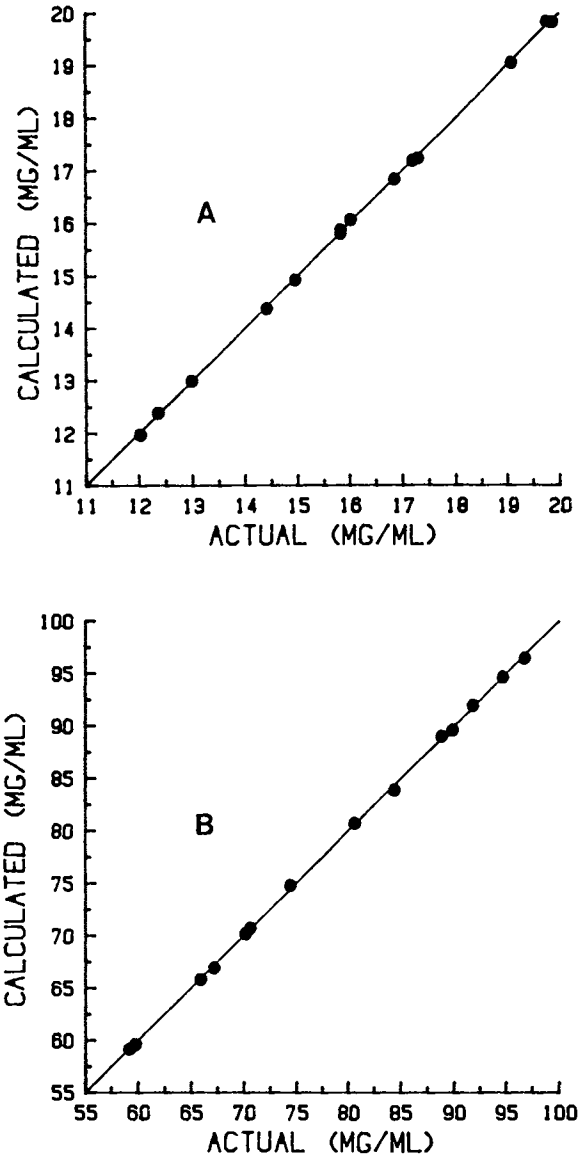

Fig. 4. Actual versus calculated calibration results for (A) trimethoprim and (B) sulfamethoxazole.

Table II. Actual Versus Calculated Values and Percentage Relative Errors for Validation Samples

Sample No.	Trimethoprim mg/ml Actual	Trimethoprim mg/ml Predicted	Trimethoprim % relative error	Sulfamethoxazole mg/ml Actual	Sulfamethoxazole mg/ml Predicted	Sulfamethoxazole % relative error
1	18.08	17.97	0.61	83.16	83.12	0.05
2	14.36	14.38	0.14	93.77	93.52	0.27
3	15.13	15.23	0.66	82.74	82.50	0.29
4	17.61	17.74	0.74	64.49	63.94	0.70
5	16.76	15.98	1.91	78.07	78.43	0.46
6	15.98	15.99	0.06	64.17	64.49	0.50

Commercial Samples

Three commercial samples of the same lot of Septra I.V. Infusion (Lot No. 741339, Burroughs Wellcome) were examined on 3 different days to evaluate the precision and ruggedness of the method, as well as to compare the results to those obtained with the HPLC method given. The results obtained (Table III) show that for trimethoprim the precision obtained was comparable for the two methods and the concentration values predicted differed by only 1.50%. For sulfamethoxazole, the concentration values were again similar (1.02%), and even though the precision was better with the HPLC technique, the FTIR method still yielded a standard deviation of less than ±1.0% of the predicted value.

The time required for individual sample analysis was also comparable, 9 min for HPLC and 10 min for FT-IR/ATR. However, the time for sample analysis by the FT-IR/ATR method can be greatly reduced by using a FTIR with a faster scanning interferometer and fast-response mercury cadmium telluride (MCT) detector. Such spectrometers are capable of collecting 100 scans/sec, reducing analysis time from minutes to seconds. Also available now is a liquid cell (QCircle, Spectra Tech, Inc.) equipped with a liquid sipping device which eliminates the need for entering the sample compartment and maintains the purge between samples. The major time investment for this technique is in the initial preparation and spectra collection of the calibration set, which for this experiment took about 8 hr. Once created, the calibration model should be valid for extended periods of time with a properly functioning spectrometer. The initial time requirement is partially offset by the need in the HPLC method for the repetitive making of mobile phase, dilutions of samples, and column equilibration times.

Table III. Comparison of FT-IR/ATR and HPLC Values for the Average of Three Commercial Samples

Component	Mean ± SD FT-IR/ATR	Mean ± SD HPLC
Trimethoprim	15.71 ± 0.18	15.95 ± 0.17
Sulfamethoxazole	81.50 ± 0.72	80.68 ± 0.21

In conclusion, this technique gave levels of accuracy and precision comparable to those of an HPLC technique and should be considered as an alternative when developing quality-control procedures for liquid pharmaceuticals.

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