DIONEX

Now sold under the Thermo Scientific brand

Thermo SCIENTIFIC

Application Note 132

Determination of Sulfur-Containing Antibiotics Using Integrated Pulsed Amperometric Detection (IPAD)

INTRODUCTION

Antibiotics are often analyzed using high performance liquid chromatography (HPLC) with absorbance detection. Official methods to assess antibiotic identity, strength, quality, and purity are described in the Code of Federal Regulations (CFR Title 21) and in the United States Pharmacopeia National Formulary (USP NF). The HPLC methods described use absorbance detectors. Non-HPLC methods are required for some antibiotics with poor chromophoric properties. For example, lincomycin is certified for identity and potency[1,2] by a method that derivatizes this analyte and uses a gas chromatograph (GC) with flame ionization detection (FID)[3]. In this time-consuming method, lincomycin is dissolved in pyridine and then derivatized using a silylating reagent. An internal standard is added after derivatization. Identity is based on retention time, and potency is based on peak area relative to a lincomycin standard. Impurity is measured as lincomycin-B (4"-etillincomycin) content, the only measurement required to certify the purity of lincomycin. GC and HPLC methods using precolumn derivatization were developed to provide determinations of lincomycin and lincomycin-B, which lack strong chromophores. However, a derivatization reaction may not go to completion, so the accuracy of these methods can be questioned. Furthermore, use of derivatization makes it impossible to accurately assess the purity of the drug because silylation is a prerequisite for detection by GC-FID, and not all impurities can be derivatized. The CFR and USP contain other examples of antibiotics with poor chromophoric properties. Consequently, it is desirable to have methods that use a simple, direct, and sensitive detection method.

Sulfur-containing antibiotics that do not contain fully oxidized sulfur can be detected electrochemically. The electrochemical detection process for sulfur compounds on noble metal electrode surfaces has been described by LaCourse[5] and Johnson[20]. During the initial detection step, sulfur compounds are preadsorbed to the oxide-free noble metal (gold) surface by a nonbonded electron pair from the sulfur group. The adsorbed sulfur moiety is then oxidized concurrently with the gold surface. A detector signal results from analyte oxidation and gold oxide formation. The IPAD waveform removes the contribution of surface oxide formation from the detector signal.

Electrochemical detectors have been successfully used on other sulfur-containing substances, for example, sulfur-containing peptides[4–7] such as glutathione. This detection has also been used for the determination of sulfur-containing amino acids (e.g., cysteine[6–8], cystine[8], methionine[6, 9], homocysteine[9–10]), and amino acid derivatives such as S,S ‘-sulfonyldiethylenedicysteine, and S,S'-thiodiethylenedicysteine[11]. Simple inorganic compounds have also been determined by this detection, such as sulfur dioxide[12], sulfite[13–14], sulfide[8, 14, 15], disulfides[8], acid-volatile sulfur[15], and thiosulfate[14]. A broad assortment of organosulfur compounds such as thiourea[6], coenzyme A derivatives[5], bis-(2 hydroxyethyl) sulfoxide, thiodiethanol, mercaptoacetic acid, dithiodiacetic acid, thioxane, bis-(2-chloroethyl) sulfoxide, dithia-6-oxaundecane-1,11-diol, and dithiane also have been analyzed by electrochemical detection[11]. Recently, this detection has successfully been used on sulfur-containing antibiotics coupled to HPLC[5,17–19].

In this Application Note we present the determination of sulfur-containing antibiotics separated by reversed-phase HPLC and detected by integrated pulsed amperometric detection (IPAD). The HPLC eluent conditions described by LaCourse and Dasenbrock[18–19] (optimized for electrochemical detection) were used in conjunction with a modified version of their waveform to separate and detect a set of sulfur-containing antibiotics representing different structural classes (Figure 1). Absorbance detection with the same eluent system was also used and the results were compared to the IPAD results. Linear range, estimated limits of detection, and precision were determined for seven antibiotics (each representing a different structural class), including one non-sulfur-containing antibiotic. The recoveries of two antibiotics from a commercial tablet formulation were determined. The feasibility of performing a dissolution study with IPAD is also described. Chemical stability studies were performed on two antibiotics, monitoring the antibiotics' peak areas and the formation of decomposition products.

EQUIPMENT

Dionex DX-500 BioLC® system consisting of:

- GP50 Gradient Pump with degas option
- ED40 Electrochemical Detector
- AD20 Absorbance Detector
- LC30 or LC25 Chromatography Oven
- AS3500 Autosampler

PeakNet™ Chromatography Workstation

For this Application Note, the AD20 cell preceded the ED40 cell.

REAGENTS AND STANDARDS

Reagents

Acetic acid, HPLC grade (J.T. Baker)

Acetonitrile, HPLC grade (Burdick & Jackson)

Deionized water, 18 MΩ-cm resistance or higher

Methanol, HPLC grade (Fisher Scientific)

Sodium hydroxide, 50% (w/w; Fisher Scientific)

Standards

Amoxicillin (Sigma)

Ampicillin, sodium salt (Fluka BioChemika)

Cefadroxil (Sigma)

Cefazolin, sodium salt (Fluka BioChemika)

Cefotaxim (Fluka BioChemika)

Cephalexin, hydrate (Sigma)

Cephaloridine, hydrate (Aldrich)

Cephalothin, sodium salt (Sigma)

Cephapirin, sodium salt (Sigma)

Cephradine (Sigma)

Cloxacillin, sodium salt, monohydrate (Sigma)

Lincomycin, hydrochloride (Sigma)

Penicillin G, potassium salt (benzylpenicillin; Fluka BioChemika)

Penicillin V (Sigma)

Sulfanilamide (Aldrich)

Sulfamethoxazole (Sigma)

Trimethoprim (Fluka BioChemika)

Samples

Sulfamethoxazole and trimethoprim tablets, USP (800 mg/160 mg; Sidmak Laboratories, Inc., East Hanover, NJ 07936)

CONDITIONS

Columns:	Vydac C8 Reversed-Phase Analytical (P/N 208TP5415)
Flow Rates:	1.0 mL/min
Injection Vol:	10 µL
Temperature:	30 °C
Eluents:	A: Water B: 500 mM sodium acetate, pH 3.75 C: 90% acetonitrile D: Methanol
On-line Degas:	30 s every 2 min
Program:	See table on page 3
Detection:	AD20: Absorbance (200, 215, 254, or 275 nm depending on the antibiotic) ED40: Integrated pulsed amperometry, gold electrode, Ag/AgCl reference electrode
Typical system operating backpressure:	8.1–10.3 MPa (1170–1500 psi)

Waveform for the ED40:

Time (seconds)	Potential (volts)	Integration (begin/end)
0.00	0.24	
0.05	0.24	Begin
0.09	1.34	
0.13	0.24	
0.17	1.34	
0.21	0.24	
0.25	1.34	
0.29	0.24	
0.33	1.34	
0.37	0.24	
0.41	1.34	
0.45	0.24	
0.49	1.34	
0.53	0.24	
0.57	1.34	
0.61	0.24	
0.65	1.34	
0.69	0.24	
0.73	1.34	
0.77	0.24	
0.81	1.34	
0.85	0.24	End
0.86	-1.50	
0.87	-1.50	
0.88	1.34	
0.89	-0.21	
1.00	-0.21	

PREPARATION OF SOLUTIONS AND REAGENTS

On-line degassing is necessary because the amperometric detector is sensitive to oxygen in the eluent. Set the pump to degas for 30 s every 2 min.

Eluents

500 mM sodium acetate, pH 3.75 (Eluent B)

Combine 57 mL of glacial acetic acid with 1.8 L water; add 50% sodium hydroxide (50% w/w) until pH is increased to 3.75 (approximately 6.0–6.8 mL). Add water until the total volume is 2.0 L. Keep the eluents blanketed under 28–69 kPa (4–10 psi) of helium at all times.

90% (v/v) acetonitrile (Eluent C)

Combine 900 mL acetonitrile with 100 mL water.

SAMPLE PREPARATION

Stock Standards

Solid antibiotic standards were dissolved in purified water to 10 g/L concentrations, correcting for the percent weight of salt and water content as specified on the label. Sulfamethoxazole and trimethoprim were not readily soluble in water and were dissolved in 70% (v/v) methanol (MeOH) to water. For determinations of linear range and lower detection limits, 10 g/L solutions of cephradine, cephapirin, sulfamethoxazole, trimethoprim, sulfanilamide, lincomycin, and ampicillin were diluted with their respective solvents to concentrations of 0.01, 0.025, 0.05, 0.075, 0.10, 0.25, 0.50, 0.75, 1.0, 2.5, 5.0, 7.5, 10, 25, 50, 75, 100, 250, 500, 750, 1000, 2500, 5000, 7500, and 10000 mg/L. The solutions were frozen at -20 °C until needed.

Program							
Eluent	**Analyte**	**Program**				**Background (IPAD)**	**Noise (Peak-to-Peak)***
100 mM Sodium Acetate (pH 3.75) with:	**Antibiotic**	**%A**	**%B**	**%C**	**%D**	**nC (Range)**	**pC (Range)**
9% Acetonitrile and 10% Methanol	Sulfamethoxazole, Trimethoprim	60	20	10	10	430–450	190–1590
9% Acetonitrile and 0% Methanol	Lincomycin	70	20	10	0	450–490	180–1300
6% Acetonitrile and 0% Methanol	Ampicillin	73.3	20	6.7	0	440–490	150–1570
4% Acetonitrile and 0% Methanol	Cephapirin, Cephradine	75.6	20	4.4	0	450–480	140–550
0% Acetonitrile and 0% Methanol	Sulfanilamide	80	20	0	0	410–460	100–340

*Measured peak-to-peak noise (IPAD) for 1-min intervals.

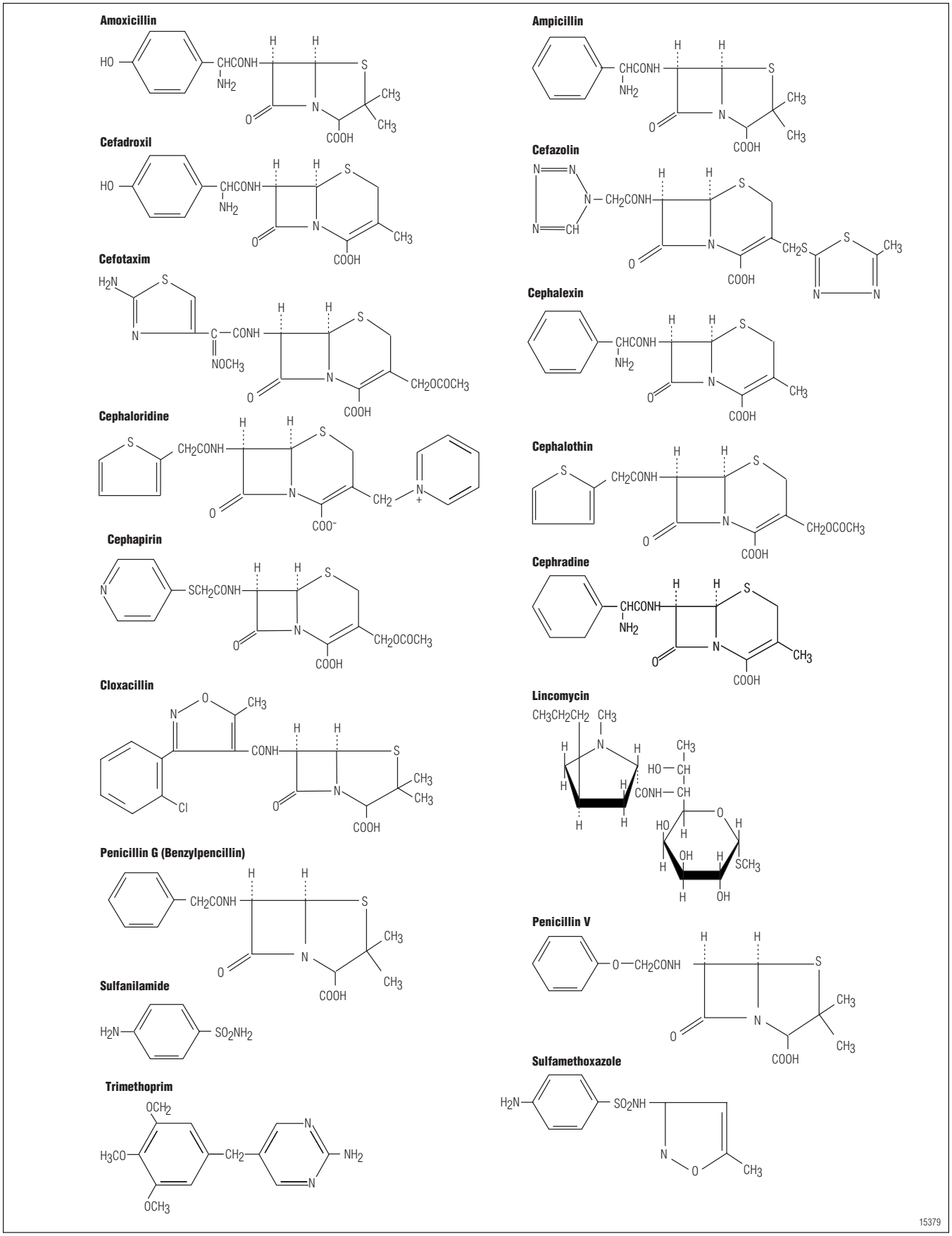

Figure 1. Chemical structures of antibiotics

Standard solutions of these antibiotics at concentrations ranging from 2.5 to 20000 times above the lower limit of detection and within the linear range were used to evaluate the precision of replicate injections.

Dissolution of Sulfamethoxazole and Trimethoprim Tablet in Water

One tablet containing 800 mg sulfamethoxazole and 160 mg trimethoprim (Sidmak Laboratories) was placed in a stainless steel mesh tea strainer and immersed in a clean, 1-L glass beaker containing 800 mL purified sterile water. The dissolution mixture was kept in constant motion using a magnetic stir bar with a rotation frequency of 70 rpm for 2 h, with 0.45 mL aliquots removed at frequent intervals between 0.5 and 10 min apart. Aliquots were diluted 3.3-fold with 1.05 mL MeOH, yielding an antibiotic sample in 70% MeOH. Insoluble particulates were removed by microcentrifugation (14000 x g, 10 min). Supernatants were directly analyzed (10-µl injection) by HPLC.

Complete Dissolution of Sulfamethoxazole and Trimethoprim Tablet in 70% Methanol

One tablet containing 800 mg sulfamethoxazole and 160 mg trimethoprim (Sidmak Laboratories) was placed in a 100-mL volumetric flask and brought to volume with 70% MeOH. The tablet in 70% MeOH was sonicated for 20 min. Some excipients listed on the product label (such as magnesium stearate, pregelatinized starch, and sodium starch glycolate) apparently did not dissolve under these conditions and were removed, with any insoluble drug, by centrifugation at 14,000 x g for 10 min. The supernatant was diluted 100-, 500-, and 1000-fold with 70% MeOH, and 10-µL aliquots were analyzed by HPLC.

RESULTS AND DISCUSSION

Selectivity

Sixteen sulfur-containing antibiotics and one non-sulfur-containing antibiotic were evaluated under different eluent conditions for their response, retention times, and detection of impurities. Appendix A shows the retention times of antibiotics with varying amounts of organic modifiers (acetonitrile and MeOH) in the mobile phase when using a Vydac C8 column flowing at 1 mL/min and a temperature of 30 °C. All sulfur-containing antibiotics tested in this Application Note are easily detected by IPAD after separation with this reversed-phase column. Sulfur-containing antibiotics that are poor chromophores (e.g., lincomycin and ampicillin) showed the most significant improvement in peak response by IPAD compared to absorbance detection in this eluent system. Figure 2A shows the chromatogram of a 1-µg injection of ampicillin (Peak 5) detected by absorbance at 215 nm. This peak is barely above the baseline noise. The ampicillin peak is large when it is detected by IPAD (Figure 2B). Furthermore, IPAD detects impurities in this antibiotic preparation that were not observed with absorbance detection (Peaks 2, 3, and 4). Similar results were obtained for lincomycin, another sulfur-containing antibiotic with poor chromophoric properties. Figure 3 shows a lincomycin chromatogram with (A) detection at 215 nm and (B) by IPAD. No peak was observed in the absorbance trace, but a significant peak was observed in the IPAD trace. Similarly,

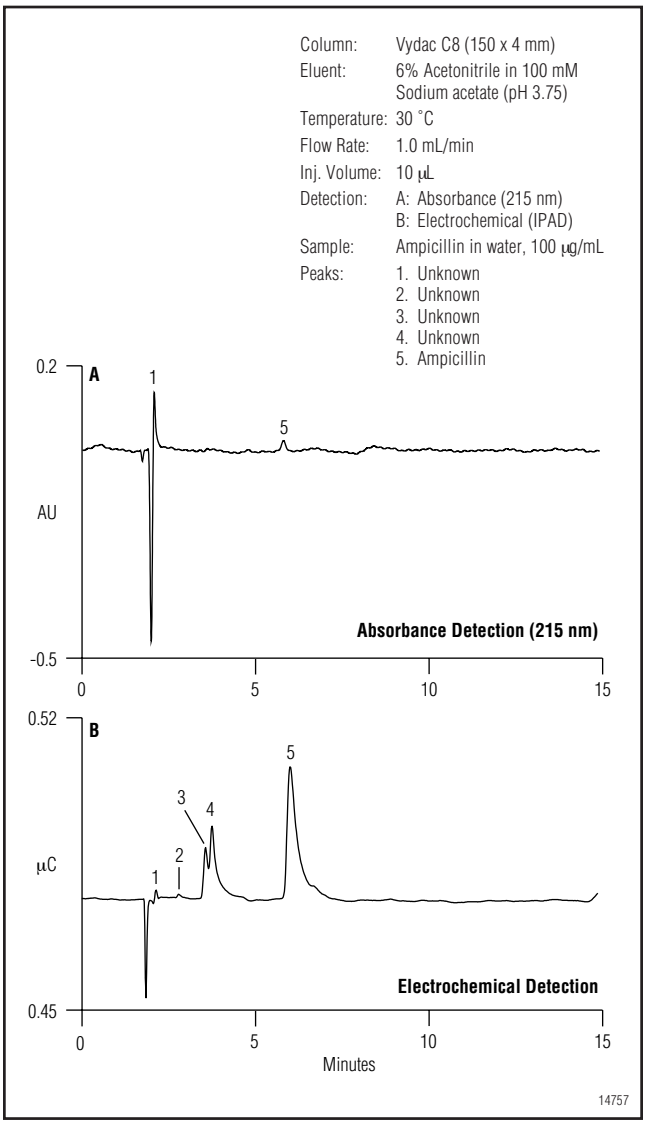

Figure 2. Ampicillin detected by (A) absorbance at 215 nm and (B) IPAD.

impurities (Peaks 2, 3, and 5) were observed in the IPAD trace. These results show that a broad spectrum of sulfur-containing antibiotics can be detected using electrochemical detection. Furthermore, sulfur-containing impurities of antibiotics may respond poorly or be undetected by absorbance, but can be easily detected by electrochemical detection.

Stability of Detector Response

To test the long-term stability of the electrode response using the waveform described in this Application Note, 100-µg/mL solutions of cephradine and cephapirin were analyzed over 64 days. Analysis was performed using a Vydac C8 reversed-phase column with 100 mM sodium acetate and 4% acetonitrile as eluent at a flow rate of 1.0 mL/min. The average peak areas obtained for 10-µL injections of these antibiotic solutions were plotted over time; Figure 4 shows those results. Both antibiotics showed a stable response over at least two months.

When the organic solvent concentration of the eluent is lowered, retention time increases and IPAD peak area increases. Increased retention time has very little effect on absorbance detector response. We hypothesize that the lower organic solvent eluent content causes less suppression of the electrochemical response.

Linearity

Ampicillin, cephradine, cephapirin, lincomycin, sulfanilamide, sulfamethoxazole, and trimethoprim standards ranging from 0.01 to 10000 mg/L (0.10 to 100000 ng in 10 µL) were injected (two or three per concentration). The response factors (peak area per ng injected) for both detectors were tabulated for each concentration and the upper limits of linearity were calculated from the concentration points at which the response factors deviated more than 10% from the linear region. Table 1 shows that absorbance detection generally had a higher linear range than electrochemical detection. For example, lincomycin was linear by absorbance detection (215 nm) up to the highest concentration tested in the study (100-µg injection), but IPAD was linear to only 0.1–0.25-µg injection. The useful calculation range can be extended to higher concentrations by using nonlinear curve-fitting algorithms.

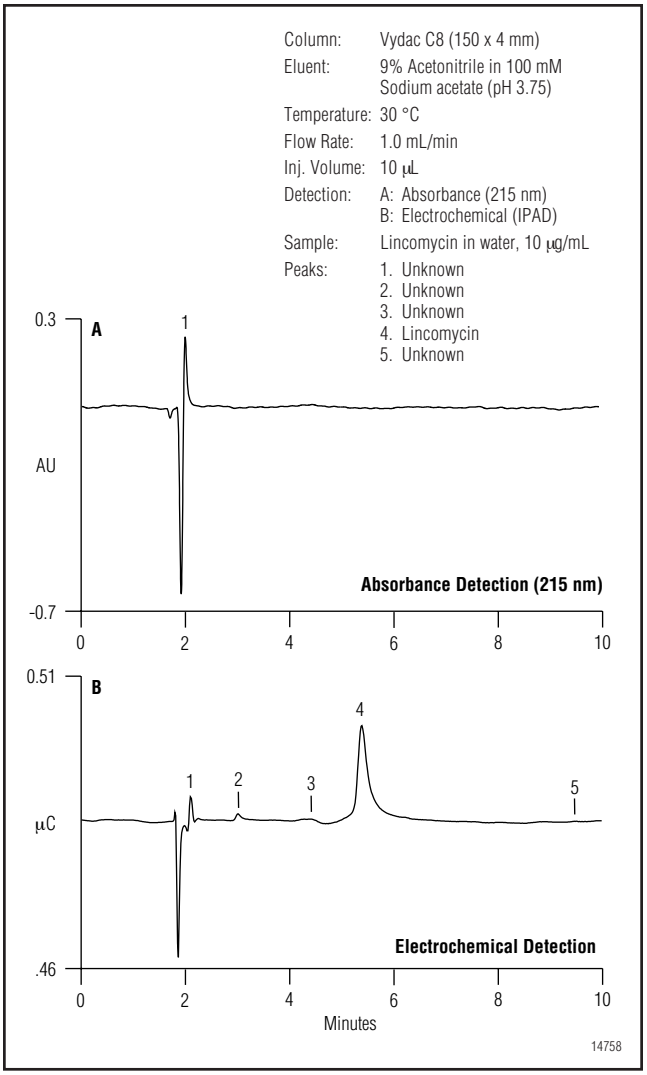

Figure 3. Lincomycin detected by (A) absorbance at 215 nm and (B) IPAD.

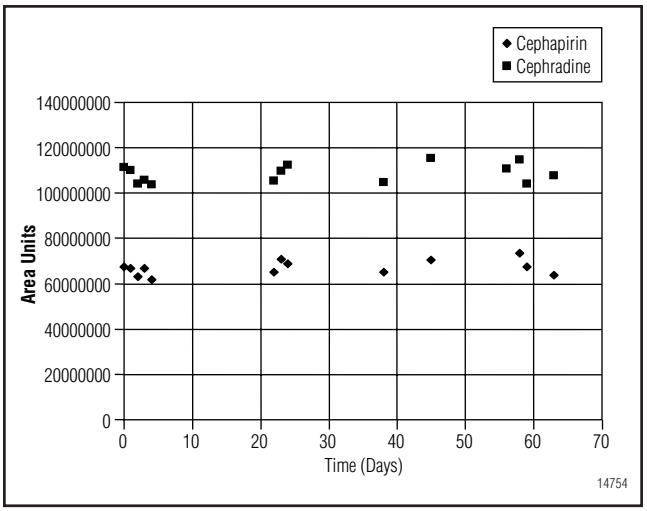

Figure 4. Stability of electrochemical response over 64 days.

Table 1 Upper Limit of Linearity

Antibiotic	Wavelength (nm)	UV Upper Limit*	IPAD Upper Limit*
Ampicillin	200	25–50 µg	1 µg
Ampicillin	254	>100 µg	1 µg
Cephapirin	254	5–7.5 µg	0.05–0.075 µg
Cephradine	254	>100 µg	0.1–0.25 µg
Lincomycin	215	>100 µg	0.1–0.25 µg
Sulfanilamide	254	1–2 µg	0.01–0.1 µg
Sulfamethoxazole	275	10–25 µg	0.025–0.05 µg
Trimethoprim	275	0.5 µg	0.05 µg

* Upper limit is defined here as the mass injected where response factor (area units/mass or slope) deviates from linearity by 10% or more.

Lower Limits of Detection

IPAD generally produced lower limits of detection (LODs) than absorbance detection in this eluent system. Estimated LOD values were calculated from the antibiotic concentrations yielding peak heights equivalent to 3 times the peak-to-peak noise. The noise was obtained from a 1-min interval of a solvent blank injection that included the retention time of the antibiotic peak. Table 2 presents the estimated LODs for detection of ampicillin, cephapirin, cephradine, lincomycin, sulfanilamide, sulfamethoxazole, and trimethoprim.

Table 2 Estimated Lower Limits of Detection

Antibiotic	Wavelength (nm)	Lower Limit of Detection*			
		UV** (µg/mL)	UV (ng Injected)	IPAD** (µg/mL)	IPAD (ng Injected)
Ampicillin	200	40	400	2	20
Ampicillin	254	10	100	2	20
Cephapirin	254	0.4	4	0.2	2
Cephradine	254	0.6	6	0.2	2
Lincomycin	215	520	5200	1	10
Sulfanilamide	254	0.04	0.4	0.01	0.1
Sulfamethoxazole	275	0.1	1	0.05	0.5
Trimethoprim	275	0.07	0.7	0.3	3

* Based on concentrations where peak heights are equal to 3 times the baseline noise.

** 10-µL injection

Nonchromophoric sulfur-containing antibiotics such as ampicillin and lincomycin showed the largest difference between the two detection methods. For example, lincomycin was detected by absorbance detection (215 nm) down to 5200 ng, and detected by IPAD down to 10 ng; hence IPAD was 520 times more sensitive under these conditions. Trimethoprim, a non-sulfur-containing antibiotic, was detected at greater sensitivity by absorbance than by IPAD. This is likely due to the absence of the sulfur atom and the presence of a chromophore. Ampicillin was evaluated at 200 and 254 nm. Although very little absorbance can be observed within the spectral region greater than about 220 nm, lower LODs were obtained at 254 nm than at 200 nm because the baseline (peak-to-peak) noise was significantly greater at lower wavelengths as a consequence of acetate absorbance at 200 nm. Neither wavelength yielded detection limits lower than those obtained by IPAD.

Because lower LODs depend on baseline noise levels and IPAD baseline noise levels in this method increase with the organic modifier content of the eluent, lower LODs are adversely affected by high levels of organic solvents in the mobile phase. To maximize detection limits, we recommend developing methods that minimize organic solvent in the eluent.

Peak Area Precision

The peak area RSDs were determined for replicate injections (n = 10) of ampicillin, cephapirin, cephradine, lincomycin, sulfanilamide, sulfamethoxazole, and trimethoprim. The results using both absorbance detection and IPAD are presented in Table 3. The precision was generally about the same for both methods. Except for ampicillin, the peak area RSD by absorbance detection ranged from 0.4 to 2%, and from 1 to 3% by IPAD. The peak area RSD for ampicillin by absorbance detection was 27% but only 3% by IPAD; this percentage is exceptionally high by absorbance detection because the concentration tested was only slightly greater than the lower limit of quantification. The results for ampicillin show the importance of high sensitivity to precision.

Table 3 Peak Area Precision (10 Injections)

Antibiotic	Conc. (µg/mL)	ng Injected*	Wavelength (nm)	% RSD UV	% RSD IPAD
Ampicillin	100	1000	200	27%	2.6%
Cephapirin	10	100	254	2.2%	2.4%
Cephradine	10	100	254	2.1%	1.5%
Lincomycin	100	1000	N/A	N/A	1.3%
Lincomycin	1000	10000	N/A	N/A	1.5%
Sulfanilamide	10	100	254	0.6%	2.2%
Sulfamethoxazole	10	100	275	1.7%	1.0%
Sulfamethoxazole	100	1000	275	0.6%	1.4%
Trimethoprim	10	100	275	2.2%	3.0%
Trimethoprim	100	1000	275	0.4%	2.6%

* 10-µL injection

Monitoring Antibiotic Stability

Some antibiotics maintained in aqueous conditions at ambient temperature (20–22 °C) chemically decompose over time. In this Application Note, the chemical stability of cephapirin and cephradine (10 µg/mL) were evaluated. Figure 5 presents cephradine peak area plotted against incubation time, detected by both absorbance detection and IPAD. Peak area loss was negligible over 69 h by both detectors. Figure 6 presents the same study conducted with cephapirin. This antibiotic showed a significant loss in peak area over time; the area units decreased at a rate of 10% per day for both detection methods. Chromatograms of fresh cephapirin (Figure 7) revealed a reasonably high level of purity based on the absence of spurious peaks. Some trace impurities were observed (Peaks 1 and 5) in both methods. After 69 h of incubation (Figure 8), two additional peaks were observed by absorbance detection (Peaks 2 and 3). Peak 4 was at or slightly above the baseline noise and could not be considered quantifiable. Four additional peaks were observed by IPAD (Peaks 2, 3, 4, and 6). Plotting the area of the extra peaks shows that both detectors can measure the same rate of change in peak areas of the new peaks, and that the higher sensitivity of IPAD for trace impurities can provide additional kinetic information not obtainable by absorbance detection (Figure 9).

Percent Recovery from Pharmaceutical Tablet Formulation

A tablet containing 800 mg sulfamethoxazole and 160 mg trimethoprim (according to the package's label) was dissolved in 100 mL of 70% MeOH:30% water. A slurry was produced that consisted of insoluble tablet excipients listed on the product label, such as magnesium stearate, pregelatinized starch, and sodium starch glycolate. Both sulfamethoxazole and trimethoprim were determined to be readily soluble in this solvent. The insoluble excipients were removed by centrifugation.

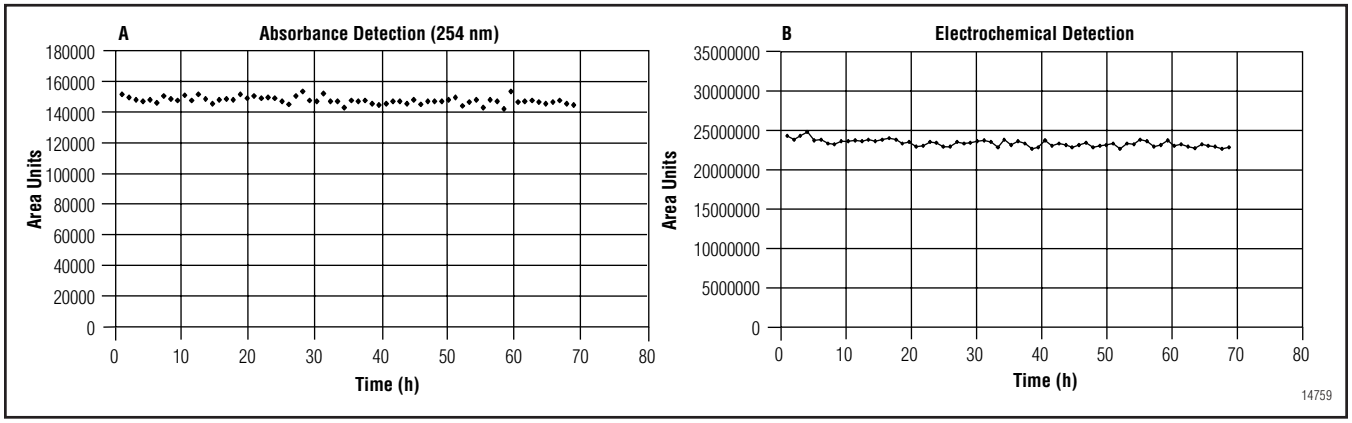

Figure 5. Monitoring cephradine (10 µg/mL) stability in water at ambient temperature by (A) absorbance detection and (B) IPAD.

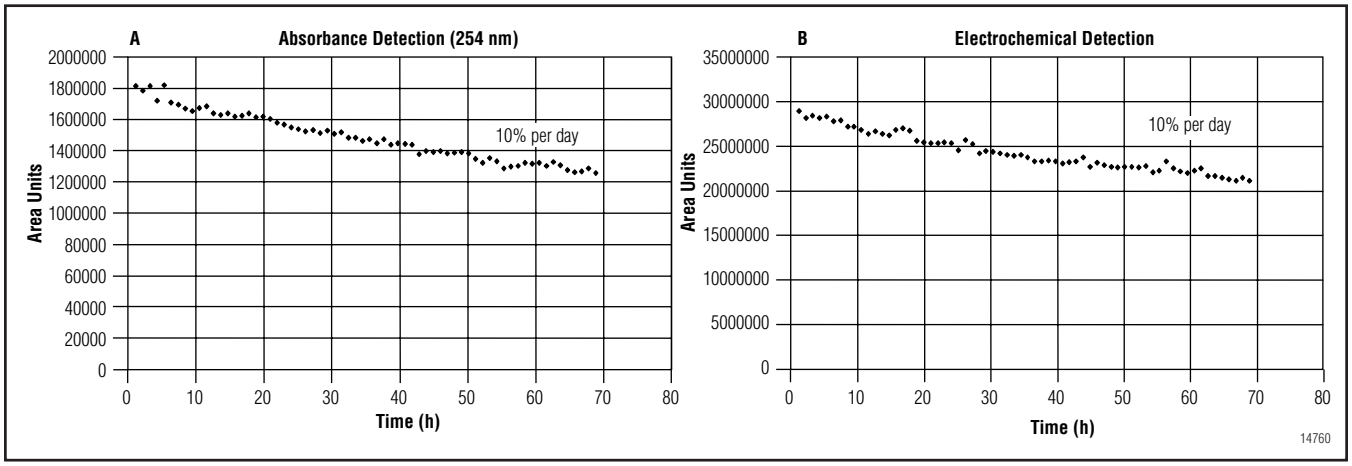

Figure 6. Monitoring cephapirin (10 µg/mL) stability in water at ambient temperature by (A) absorbance detection and (B) IPAD.

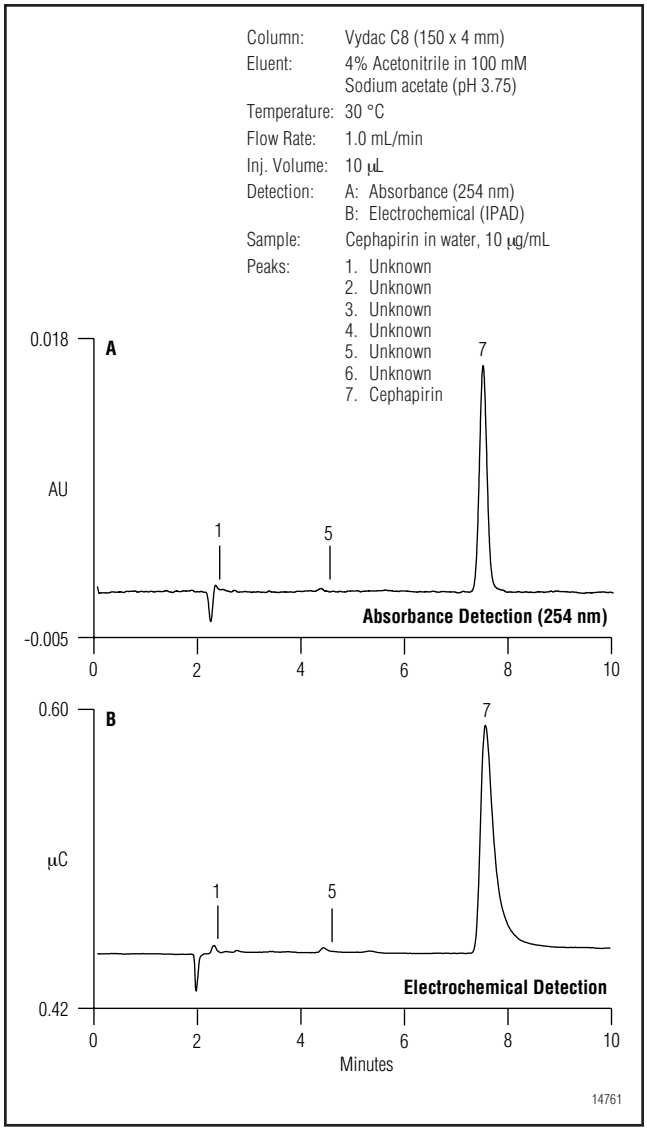

Figure 7. Chromatograms of cephapirin by (A) absorbance detection and (B) IPAD.

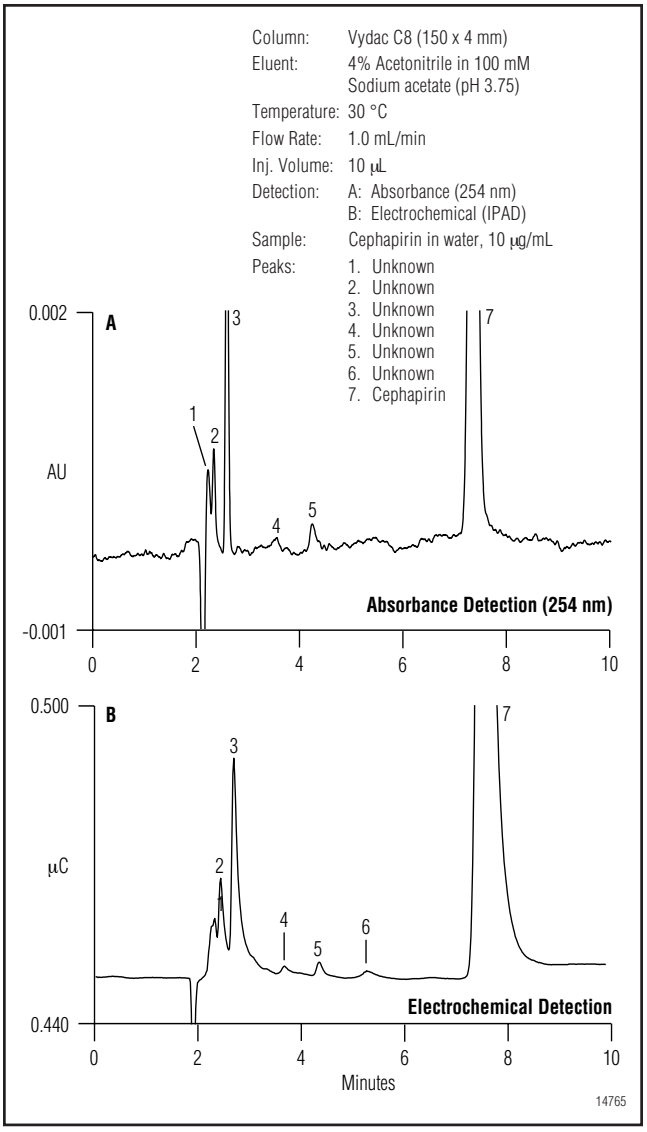

Figure 8. Chromatograms of cephapirin after 69-h incubation in water at ambient temperature by (A) absorbance detection and (B) IPAD.

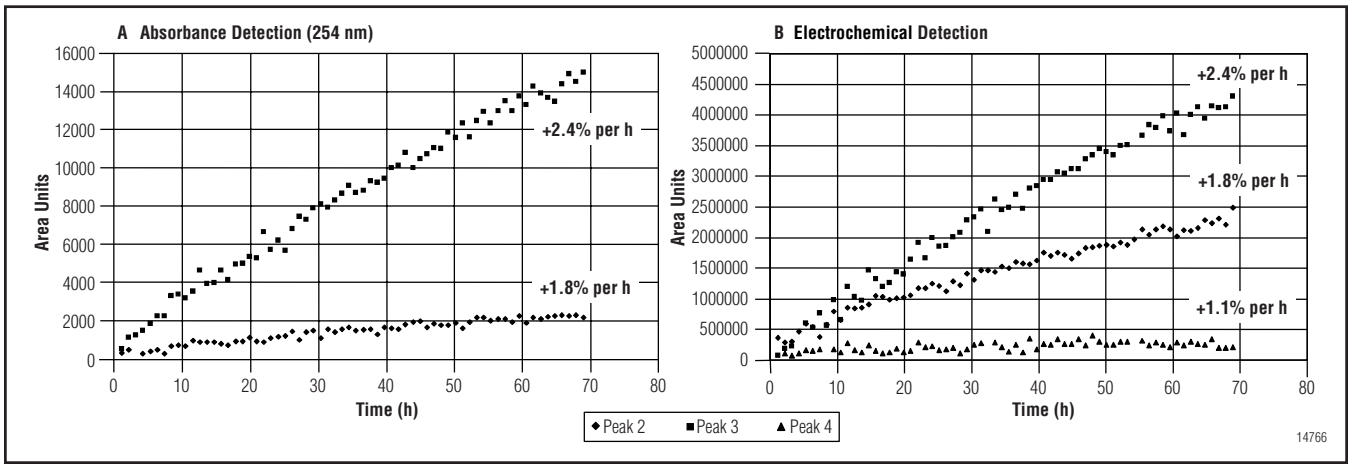

Figure 9. Monitoring cephapirin (10 µg/mL) decomposition products by (A) absorbance detection and (B) IPAD.

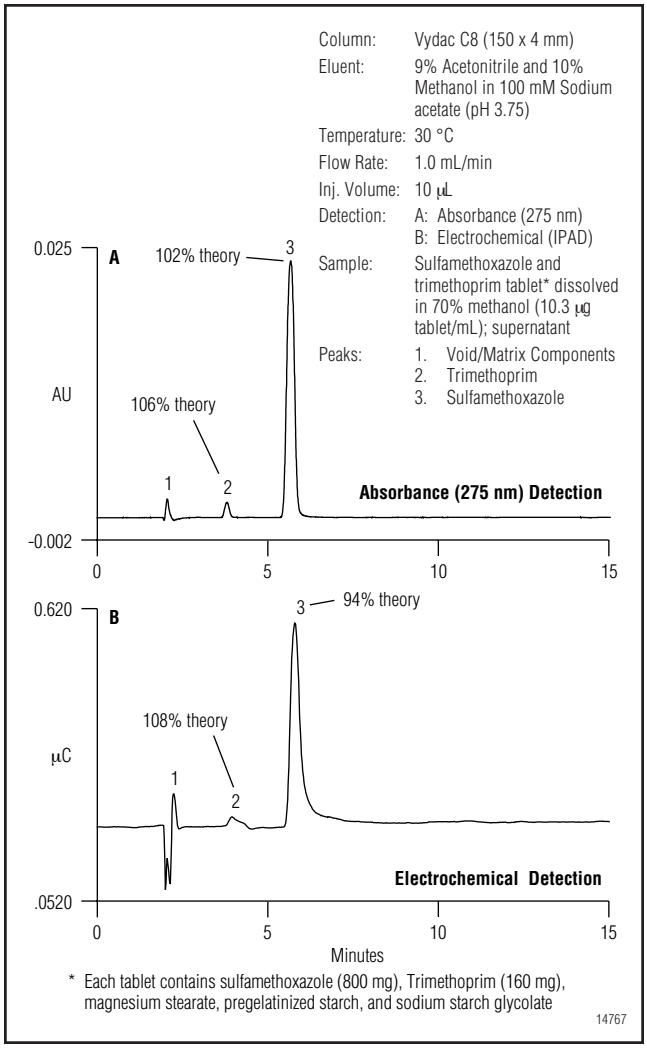

Figure 10. Recovery of sulfamethoxazole and trimethoprim from a tablet formulation by (A) absorbance detection and (B) IPAD.

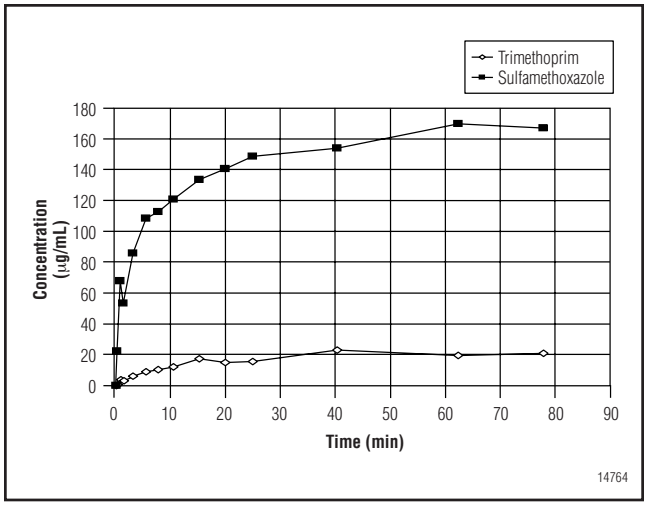

Figure 11. Determination of the dissolution of sulfamethoxazole and trimethoprim from a tablet formulation by IPAD.

The peak areas obtained for both antibiotics were related to standard calibration curves to determine their concentrations. Chromatograms produced by absorbance detection and IPAD are presented in Figure 10. The measured value of sulfamethoxazole recovered from the tablet by absorbance detection at 275 nm was 102% of the label value and 94% of the label value by IPAD. Trimethoprim measured by absorbance detection yielded 106% of the amount on the label, and 108% by IPAD. These recoveries demonstrate comparable accuracy for the two detection methods.

Dissolution of a Pharmaceutical Tablet Formulation in Water

We also investigated the feasibility of conducting a drug dissolution study using electrochemical detection. The sulfamethoxazole-trimethoprim tablet was used to study the kinetics of (1) dissolution in a nonoptimal solvent, (2) the drugs' release from insoluble excipients present in the tablet formulation, and (3) their release from the stainless steel wire mesh used to contain the tablet during dissolution. Neither sulfamethoxazole nor trimethoprim is readily soluble in water. Magnesium stearate, pregelatinized starch, and sodium starch glycolate are present in the tablet formulation as binders and, to some extent, facilitate the rate at which the drugs are released upon ingestion. In this study, a stainless steel mesh strainer was used to contain the tablet during dissolution. Solubility in water, release from an insoluble matrix, and release from the stainless steel container are all expected to participate in the measured release kinetics of the two drugs. To assure that all the released drugs were solubilized for analysis, aliquots of the suspension collected at designated time points were diluted in sufficient MeOH to produce a 70% MeOH solution and the insoluble particulates (excipient material or drugs) were then removed by centrifugation. Figure 11 presents the results of the dissolution study and shows that sulfamethoxazole reaches a steady state after about 50–60 min, and trimethoprim after 30–40 min. About 60% of the sulfamethoxazole was dissolved upon reaching its steady state, but only about 30% of the trimethoprim was dissolved after 30 min. These results were not collected by officially recognized dissolution procedures as described by the FDA or USP, and therefore should not be regarded as an accurate depiction of true kinetic behavior of this drug formulation. The purpose of the study was to show the feasibility of using IPAD for conducting drug measurements in these types

of studies. It is also expected that under circumstances where the excipients of a formulation are both chromophoric and soluble in the dissolution solvent, IPAD may be favorable in revealing the levels of either drug or drug-related impurities by reducing the level of interferences that are absorbing but electrochemically inactive.

CONCLUSION

IPAD is a good detection choice for nonchrom-ophoric sulfur-containing antibiotics. Some impurities resulting from the antibiotic manufacture or chemical decomposition may be detected better by IPAD than by absorbance. The specificity of IPAD for substances that can be oxidized using the electrode potentials selected for this study helps reduce interferences from chromophoric matrix ingredients or eluent components. IPAD may exhibit lower detection limits for sulfur-containing antibiotics and thus could be considered an alternative detection method for these compounds.

REFERENCES

1. Code of Federal Regulations (CFR) Title 21; Part 453, Subpart A–Bulk Drugs, Section 30 "Lincomycin hydrochloride monohydrate," Subsection (a) "Requirements for Certification." U.S. Department of Health and Human Services. Food and Drug Administration. U.S. Government Printing Office: Washington, DC, Vol. 5.
2. Code of Federal Regulations (CFR) Title 21; Part 453, Subpart B–Oral Dosage Forms; Section 130 "Lincomycin hydrochloride monohydrate capsules," Subsection (a) "Requirements for Certification." U.S. Department of Health and Human Services. Food and Drug Administration. U.S. Government Printing Office: Washington, DC, Vol. 5.
3. Code of Federal Regulations (CFR) Title 21; Part 436, Subpart F–Chemical Tests for Specific Antibiotics; Section 306 "Lincomycin gas liquid chromatography." U.S. Department of Health and Human Services. Food and Drug Administration. U.S. Government Printing Office: Washington, DC, Vol. 5.
4. "Determination of Glutathione in Cultured Mammalian Cells with Integrated Amperometry." Application Note 110; Dionex Corporation.
5. LaCourse, W.R.; Owens, G.S. "Pulsed Electrochemical Detection of Thio-Compounds Following Microchromatographic Separations." *Anal. Chim. Acta* **1995**; 307, 301–319.
6. Vandeberg, P.J.; Johnson, D.C. "Pulsed Electrochemical Detection of Cysteine, Cystine, Methionine and Glutathione at Gold Electrodes Following Their Separation by Liquid Chromatography." *Anal. Chem.* **1993**; 65, 2713–2718.
7. Van Riel, J.A.M.; Olieman, C. "Selective Detection in Reversed-Phase High-Performance Liquid Chromatography of Tyrosine, Tryptophan and Sulfur-Containing Peptides by Pulsed Amperometry at Platinum." *Anal. Chem.* **1995**; 67, 3911–3915.
8. Vandeberg, P.J.; Johnson, D.C. "Comparison of Pulsed Amperometric Detection and Integrated Voltammetric Detection for Organic Sulfur Compounds in Liquid Chromatography." *Anal. Chim. Acta* **1994**; 290, 317–327.
9. Cole, D.E.C.; Lehotay, D.C.; Evrovski, J. "Simplified Simultaneous Assay of Total Plasma Homocysteine and Methionine by HPLC and Pulsed Integrated Amperometry." *Clin. Chem.* (Washington D.C.) **1998**; 44, 188–190.
10. Evroski, J.; Callaghan, M.; Cole, D.E.C. "Determination of Homocysteine by HPLC with Pulsed Integrated Amperometry." *Clin. Chem.* (Winston-Salem, NC) **1995**; 41, 757–758.
11. Clark, A.J. "Determination of Organosulfur Compounds and Amino Acid-Mustard Conjugates by Liquid Chromatography with Amperometric Detection." *Anal. Proc.* (London) **1993**; 30, 355–357.
12. Cardwell, T.J.; Cattrall, R.W.; Chen, G.M.; Iles, P.J.; Hamilton, I.C.; Scollary, G.R. "Determination of Sulfur Dioxide in White Wines by Flow Injection with Electrochemical Detection." *Electroanalysis* (New York) **1991**; 3, 859–863.
13. Wagner, H.P.; McGarrity, M.J. "Determination of Sulfite in Beer Using Ion-Exclusion Chromatography and Pulsed Amperometric Detection." *J. Am. Soc. Brew. Chem.* **1992**; 50, 1–3.
14. Okutani, T.; Yamakawa, K.; Sakuragawa, A.; Gotoh, R. "Determination of a Micro-Amount of Sulfite by Ion Chromatography with Amperometric Detection After Co-Precipitation with Basic Zinc Carbonate." *Anal. Sci.* **1993**; 9, 731–734.
15. Steinmann, P.; Shotyk, W. "Ion Chromatography of Organic-Rich Natural Waters from Peatlands IV. Dissolved Free Sulfide and Acid-Volatile Sulfur." *J. Chromatogr. A.* **1995**; 706, 287–292.
16. Vandenberg, P.J.; Kowagoe, J.L.; Johnson, D.C. "Pulsed Amperometric Detection of Sulfur Compounds: Thiourea at Gold Electrodes." *Anal. Chim. Acta* **1992**; 260, 1–11.

17. LaCourse, W.R.; Dasenbrock, C.O. "High Performance Liquid Chromatography-Pulsed Electrochemical Detection for the Analysis of Antibiotics," *Adv. Chromatogr.* **1998**; 38, 189–232.
18. Dasenbrock, C.O.; LaCourse, W.R. "Pulsed Electrochemical Detection of Sulfur-Containing Antibiotics Following High Performance Liquid Chromatography." *J. Pharm. Biomed. Anal.* **1999**; 19, 239–252.
19. Dasenbrock, C.O.; LaCourse, W.R. "Assay for Cephapirin and Ampicillin in Raw Milk by High Performance Liquid Chromatography-Integrated Amperometric Detection." *Anal. Chem.* **1998**; 70, 2415–2420.
20. Johnson, D.C.; LaCourse, W.R. "Liquid Chromatography with Pulsed Electrochemical Detection at Gold and Platinum Electrodes." *Anal. Chem.* **1990**; 62, 589A–597A.

LIST OF SUPPLIERS

Aldrich Chemical Company Inc., 1001 West St. Paul Avenue, P.O. Box 355, Milwaukee, Wisconsin 53233, U.S.A. Tel: 800-558-9160.

Burdick & Jackson, 1953 South Harvey Street, Muskegon, Michigan 49442, U.S.A. Tel: 800-368-0050.

Fisher Scientific, 711 Forbes Avenue, Pittsburgh, PA 15219-4785, U.S.A. Tel: 800-766-7000.

Fluka BioChemika, Fluka Chemie AG, Industriestrasse 25, Buchs 9471, Switzerland. Tel: 081-755-25-11.

J.T. Baker Inc., 222 Red School Lane, Phillipsburg, New Jersey 08865, U.S.A. Tel: 800-582-2537.

Sigma Chemical Company, P.O. Box 14508, St. Louis, Missouri 63178, U.S.A. Tel: 800-325-3010.

100 mM Sodium acetate (pH 3.75) with:	Sulfanilamide	Amoxicillin	Cefadroxil	Cephapirin	Cephalexin	Ampicillin	Cephaloridine	Cefotaxim	Cefazolin	Cephradine	Lincomycin	Trimethoprim	Sulfamethoxazole	Cephalothin	Penicillin G (Benzylpenicillin)	Penicillin V	Cloxacillin
9% Acetonitrile and 40% MeOH	1.9			2.1		2.0	2.0	1.9	1.9	2.1	2.1	2.0	1.9	2.3	2.3	2.7	2.9
9% Acetonitrile and 30% MeOH	1.9			2.2		2.1	2.1		2.0	2.3	2.3	2.1	2.3	2.9	3.2	4.5	5.7
9% Acetonitrile and 20% MeOH	2.0	2.1		2.3	2.3	2.2	2.4	2.1	2.1	2.6	2.6	2.5	3.0	4.0	4.8	8.2	13.6
9% Acetonitrile and 10% MeOH	2.1	2.1		2.5	2.6	2.6	3.1	2.5	2.5	3.0	3.3	3.4	5.1	6.6	9.8	20.1	>30
9% Acetonitrile and 0% MeOH	2.3	2.2		3.3	3.6	3.5	5.2	4.0	4.2	4.7	5.3	6.6	11.5	18.3	25.1	>30	
6% Acetonitrile and 0% MeOH	2.4	2.4		5.1	6.0	6.2	8.3	7.5	8.4	8.5	9.5	12.3	18.4	>30	>60		
5% Acetonitrile and 0% MeOH	2.5	2.4	2.6	6.5	7.6	7.7	10.0	9.6	11.0	11.2	11.8	15.3	21.3				
4% Acetonitrile and 0% MeOH	2.5		2.9	7.5		10.5	12.9	13.9	16.2	14.6	16.0	21.3	26.2				
3% Acetonitrile and 0% MeOH	2.6	2.5	3.4	12.4	13.5	15.5	17.8	22.3	26.2	>30	22.6	31.9	33.6				
2% Acetonitrile and 0% MeOH	2.7	3.4	4.0	16.4	19.9	22.2	23.7	34.7	40.4		29.6	46.1	41.0				
1% Acetonitrile and 0% MeOH	2.9	3.5	5.3	33.6	35.6	39.1	38.5	>60	>60		48.5	>60	58.1				
0% Acetonitrile and 0% MeOH	3.0	4.0	6.3	>60	>60	54.1	52.0				>60		>60				

Identity based on major eluting peak

Appendix 1. Selectivity of sulfur-containing antibiotics on a Vydac C8 Column.

Dionex Corporation
1228 Titan Way
P.O. Box 3603
Sunnyvale, CA
94088-3603
(408) 737-0700

Dionex Corporation
Salt Lake City Technical Center
1515 West 2200 South, Suite A
Salt Lake City, UT
84119-1484
(801) 972-9292

Dionex U.S. Regional Offices
Sunnyvale, CA (408) 737-8522
Westmont, IL (630) 789-3660
Houston, TX (281) 847-5652
Atlanta, GA (770) 432-8100
Marlton, NJ (856) 596-06009

Dionex International Subsidiaries
Austria (01) 616 51 25 *Belgium* (32) 3-353 42 94 *Canada* (905) 844-9650 *China* (852) 2428 3282 *Denmark* (45) 36 36 90 90
France 01 39 30 01 10 *Germany* 06126-991-0 *Italy* (06) 66 51 50 52 *Japan* (06) 6885-1213 *The Netherlands* (0161) 43 43 03
Switzerland (062) 205 99 66 *United Kingdom* (01276) 691722
* Designed, developed, and manufactured under an NSAI registered ISO 9001 Quality System.

LPN 1126-01 2M 6/02