MINIREVIEW

Recent Analytical Methods for Cephalosporins in Biological Fluids

ROGER D. TOOTHAKER, D. SCOTT WRIGHT, AND LAWRENCE A. PACHLA*

Department of Pharmacokinetics/Drug Metabolism, Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, Michigan 48105

INTRODUCTION

This review surveys cephalosporin analytical methodology cited in Analytical Abstracts between January 1980 and July 1986. The article is exclusively directed toward the analysis of cephalosporins in biological matrices. Because of the large number of references that appeared as individual methods or as part of clinical and pharmacological studies, it is possible to cite only a representative sampling of the works published. More comprehensive reviews of available methodology for these drugs in formulated or bulk drugs have appeared (27, 27a, 28). The review covers methods for cefaclor, cephapirin, cefazolin, cefoxitin, cephalothin, cephaloridine, cephalothin, cefazolin, cefradine, cefmetazole, cefonicid, cefotetan, cefotiam, cefoxitin, ceftroxadine, cefamandole, cefixime, cefmenoxime, cefoperazone, cefotaxime, cefprozil, cefotolin, ceftazidime, ceftriaxone. The cephalosporins are discussed in the above-cited order.

Cephalosporin methods for biological samples predominantly rely on precipitation or extraction cleanup steps followed by reversed-phase high-performance liquid chromatography (RP-HPLC) with UV detection. Differences in methods for each drug are summarized, and summaries of other published assay methods are given. Many of the methods are applicable to determining a multitude of cephalosporins. For these cases, the analytical method will be described for only one of the drugs unless methodological differences dictate to the contrary. There are appropriate indications in the text of where more detailed methodological descriptions can be found.

ANALYTICAL METHODS

Cefaclor. Two methods introduced for cefaclor were based on protein precipitation. In the first method, the supernatant was directly quantified by RP-HPLC after precipitation with cold methanol-0.1 M sodium acetate (65). Recovery was excellent, and the detection limit was 0.2 μg/ml. The second method involved methanol deproteinization, evaporation of the supernatant, and separation on a μBondapak column (52). Calibration curves were linear between 0.5 and 30 μg/ml for urine and plasma samples. Another set cefaclor method involved extraction with acidic chloroform-butanol (3:1) (3). Chromatography included a phenyl column and gradient elution with an aqueous methanol-ammonium acetate mobile phase. Observed interferences from theophylline and chloramphenicol can be avoided by including an additional extraction step or by changing the mobile phase pH.

Cephapirin. Cephapirin has been quantified by either direct precipitation methods (21, 65) or column isolation procedures (25). One precipitation method relied on mixing equal volumes of serum with internal standard (4-nitroacetanilide in 100 μl of acetonitrile) followed by centrifugation and direct injection of a 25-μl sample onto a C18 column (21). Detection was at 254 nm and calibration graphs were rectilinear up to 100 mg/ml, with recoveries of >90%. The second method was based on precipitation with methanolic sodium acetate followed by quantitation on a μBondapak C18 column using a mobile phase consisting of 0.01 M acetate in acetonitrile-methanol (24:1). The method had a detection limit in the range of 0.2 to 1.0 μg/ml and recoveries greater than 95%. Another method relied on ion-exchange isolation columns for sample cleanup (25). Minimal interference from endogenous sample components, maximal isolation recoveries, and method specificity were established for the potential presence of 22 commonly coadministered drugs.

Cefazolin. Method for cefazolin in serum and urine used RP-HPLC, postcolumn derivatization, and fluorescent detection with excitation at 585 nm and emission at 480 nm (19). Serum or urine samples were deproteinized with 6% trichloroacetic acid before chromatographic quantitation. The use of a packed-bed fluorescamine reactor provided rectilinear calibration curves for 10 to 100 μg of drug per ml and recoveries of approximately 100%. A similar method for cefzolin in serum and urine used UV detection at 254 nm (18). A fluorimetric method appeared for cefazolin in plasma, serum, and urine samples (47). The method involved acid hydrolysis and fluorescent product formation using hydrogen peroxide. The product was extracted into acetone-chloroform between pH 5.5 and 6.5 and fluorometrically quantified using excitation and emission wavelengths of 340 and 420 nm.

Cefotaxime. Two HPLC methods appeared for quantifying cefotaxime (11, 44). Both methods required separation and quantitation using C8 RP columns and phosphoric acid-methanol mobile phases. The first method (11) gave excellent correlation coefficients with a microbiological assay of 0.968 for sera after oral dosing and 0.944 after intravenous dosing. Recoveries were 92.5 and 95% for 2 and 10 μg/ml, respectively. The second method also agreed with a microbiological assay. This procedure emphasized the determination of cefotaxime, cefuroxime, cefmenoxime, and cefoxitin. The method involved the use of two mobile phases, one of which included methanol (51, 72), methanolic sodium acetate (65), and trichloroacetic acid (44, 68). Trichloroacetic acid precipitation methods require an ether
acetate extraction that interfered. The cefatrizine chromatographic method is also applicable for cephradine (47). A major drawback of the method involves the choice of the mobile phase. Acidic hydrolysis of cephalothin produces a degradation product that has a well-defined polarographic reduction wave. The wave is proportional to concentration and thus forms the basis for the electroanalytical measurement of cephalothin in plasma (55). Recoveries of 98% (RSD [relative standard deviation] = 2%) were reported. Unfortunately, the technique lacks sensitivity (detection limit, 40 μg/ml).

Cephadroxil. Two RP-HPLC methods were reported for cephadroxil in plasma or urine during this review period (36, 65). One of the methods was applied to rat urine samples contaminated with feces and diet pellets (36). Sample pretreatment with ethyl acetate preceded extraction of cephadroxil with methanol-0.01 M H3PO4-H2PO4 buffered to pH 7.5. The second method involved a methanolic protein precipitation and served as the basis for the general analysis of several cephalosporins. Analytical conditions are described in the cephalixin section.

Cephalothin. A variety of cephalothin methods were reported for plasma and urine samples. The simplest plasma method relied on methanolic protein precipitation followed by RP quantitation (65). Another method was based on ethyl acetate extraction of acidified plasma samples or direct injection of urine samples (20). Quantitation was achieved using a customized stationary phase with a mobile phase containing 9% aqueous ethanol and 0.2% ammonium acetate. The acidic chloroform-butanol (3:1) extraction method described for cefaclor is also applicable to cephalothin (3). An acidic chloroform-pentanol (3:1) extraction with back-extraction into neutral phosphate buffer was reported for cephalothin plasma analysis (17). This method used isocratic RP-HPLC. Urine samples were directly injected into the HPLC system.

A UV spectrophotometric method was introduced for quantifying this drug in serum or diluted urine (61). The method involves trichloroacetic acid precipitation and hydrolysis. The hydrolytic product was condensed with ninhydrin and then isolated with chloroform. The reported limit of detection was 1 μg/ml, with a precision of less than 4%.

Cefazolin. Plasma deproteinization by RP-HPLC has been used to measure cefazolin (65). Acetonitrile was used as the serum protein precipitant in a method which used a Radial-PACK C18 HPLC column (42). Another method involved an extraction with ethyl acetate of acidified serum samples; separation was achieved on a modified silica column with a mobile phase of 9% aqueous ethanol containing 0.2% ammonium acetate (20). Urine samples were directly analyzed.

Chloroform-butanol (3:1) was used to extract cefazolin from acidified serum samples in a previously described method (3). The acidic chloroform-pentanol (3:1) extraction method described for cephalothin has also been used for cefazolin (17). HPLC conditions were suitable for direct injection of urine samples. An RP-HPLC method for serum and pelvic tissue samples used acetonitrile protein precipitation followed by a methylene chloride wash (6). Drug recovery from pelvic tissue was greater than 95%. Cefazolin has been measured in cancellous and cortical bone samples after vibration mill homogenization to a particle size of 0.3 to 2.4 μm and then quantified using a standard bioassay (58). Observed differences in recoveries for the two sample types were attributed to the magnitude of the required homogenization times.

Cephradine. Urine samples or deproteinized serum samples can be assayed for cephradine by RP-HPLC using a methanol-phosphate buffer mobile phase (32). The method is capable of quantifying cephradine at levels of less than 100 μg/ml in 15- to 20-μl samples of urine or deproteinized serum using a μBondapak C18 column and a methanol-0.01 M (pH 6.8) phosphate buffer mobile phase. Fluorimetry has also been used to measure cephradine in serum and synovial fluid samples (9). The method was linear from 6 to 500 μg/ml, with a mean recovery of 97.1%. Interference by penicillin and cephalothin was negligible; however, gentamicin and tobramycin significantly interfered. The cefatrizine fluorimetric method is also applicable for cephradine (47). A major drawback of the method involves the choice of the mobile phase. Acidic hydrolysis of cephalexin produces a degradation product that has a well-defined polarographic reduction wave. The wave is proportional to concentration and thus forms the basis for the electroanalytical measurement of cephaloxin in plasma (55). Recoveries of 98% (RSD [relative standard deviation] = 2%) were reported. Unfortunately, the technique lacks sensitivity (detection limit, 40 μg/ml).

Cefmetazole. Two RP-HPLC methods for cefmetazole have been presented. In one, serum was deproteinized with 5% trichloroacetic acid in methanol and centrifuged, and a sample of the supernatant was quantified by C18 RP chromatography (64). The quantification limit was 0.4 μg/ml, and results correlated with those of a microbiological assay. In the second method, plasma samples were deproteinized using a mixture of phosphoric acid and acetonitrile and centrifuged, the supernatant was evaporated, and the residue was reconstituted with water (53). The recovery and quantitation limit were 90% and 0.1 μg/ml, respectively.

Cefonicid. An RP-HPLC method has appeared for quantifying cefonicid in plasma and urine (15). Samples were purified using solid-phase extraction and quantified using RP-HPLC. The assay was linear from 0.05 to 1 μg/ml in plasma and from 1 to 50 μg/ml in urine.

Cefotetan. A cefotetan epimer in human plasma and urine (38) was analyzed using RP-HPLC and isocratic elution with acetonitrile containing monobasic sodium phosphate and tetrabutylammonium bromide. Serum samples were deproteinized with acetonitrile and washed with dichloromethane, whereas urine samples were diluted 10-fold with 50 mM monobasic sodium phosphate. A linearity range of 1.25 to 100 μg/ml was reported, and the method was suitable for pharmacokinetic studies.

Cefotiam. Two RP-HPLC methods were reported during this review period for quantifying cefotiam and other cephalosporins after deproteinization (37, 44). An LC method involved the simultaneous determination of cefotiam and cefsulodin in serum and bone marrow (75). Samples were precipitated with acetonitrile, and separation was achieved using a μBondapak column. The mobile phase consisted of 35% methanol and 5 mM tetrabutylammonium phosphate, and the A280 was monitored. Calibration curves were rectilinear from 10 to 100 μg/ml, and recoveries were greater than 98%.

Cefoxitin. Cefoxitin has been analyzed in a variety of biofluids (e.g., serum, plasma, cerebrospinal fluid [CSF], bile, and urine). All analyses employed isocratic RP-HPLC. One method involved serum deproteinization with methanolic 5% trichloroacetic acid; the supernatant was directly quantified on a μBondapak column with a mobile phase containing acetonitrile, acetic acid, and potassium mono-
phosphate (74). The method had a quantitation limit of 2 µg/ml and correlated well with a microbiological assay. A similar method for analyzing cefoxitin in serum and CSF used ethanol deproteinization for serum and CSF samples (70). The supernatant was analyzed by RP-HPLC. Quantitation limits of 1 µg/ml were reported for both sample types. Acetonitrile has also been used as a deproteinization agent in a method for determining cefoxitin and other cephalosporins in serum (21). Recovery and intra- and interday precision were excellent. A custom RP stationary phase was used in analyzing cefoxitin in serum and urine (20). Serum sample cleanup consisted of an acidic ethyl acetate extraction, whereas urine was assayed directly. Cefoxitin has been analyzed by methods already described (17, 25, 53, 65).

Two methods appeared for measuring cefoxitin in bile. In the first, bile samples were diluted with 2 M sodium acetate and cefoxitin was isolated on a Carbopak B column (41). After elution with methanol, the eluant was evaporated and reconstituted with methanol, and a sample was assayed by RP-HPLC. Recovery ranged from 96.7 to 100.5%, and the quantitation limit was 1 µg/ml. In the second method, acetate buffer (pH 4.6) was added to a bile sample, the mixture was centrifuged, and a sample was quantified directly on a C8 RP column (56).

Cefuroxime. A selective fluorimetric method was developed for cefuroxime in body fluids (2). Acidified samples (HCl) were heated, cooled, and neutralized with NaOH. The samples were then reextracted and fluorimetrically quantified at 440 nm (excitation, 375 nm). The method correlated well with a standard microbiological assay. In another method, urine and deproteinized serum samples were neutralized before analysis (16). The detection limit for both sample types was 0.5 µg/ml. Hekster et al. (33) compared an LC (C8) assay with a microbiological assay for determining cefuroxime in rabbit plasma. The chromatographic method gave a detection limit of 0.5 µg/ml using spectrophotometric detection and correlated well (r = 0.991) with a microbiological assay. Other chromatographic methods, that are discussed in the cephalothin, cephotiam, and cefoxitin sections, have been used to analyze cefuroxime in serum, plasma, or urine (17, 20, 44).

Cefamandole. Both gradient and isocratic LC methods were used for the analysis of cefamandole. Serum penicillin, cefamandole, and other cephalosporins were simultaneously determined using gradient LC. The drugs were extracted with chloroform-butanol (3:1), the extract was evaporated to dryness, and the residue was reconstituted in 10 mM ammonium acetate and injected onto a phenyl analytical column (3). A linear gradient was performed using methanol and 10 mM ammonium acetate (pH 4.2). Cefamandole recovery was 98%. An isocratic method was used to analyze cefamandole in serum (7). Samples were extracted with acetonitrile-methylene chloride, and the extract was directly analyzed using a C8 system. The method gave a detection limit of 0.05 µg/ml and correlated well (r = 0.974) with a microbiological assay. Other isocratic methods for cefamandole have been described above in the cefaclor (65), cephalothin (17), and cefoxitin (20, 21) sections.

Cefixime. An automated column-switching LC method was used to analyze cefixime in human serum and urine (69). Deproteinized serum or diluted urine samples were partially purified using an anion-exchange column and then concentrated on a 1-cm C18 precolumn before switching to a 15-cm analytical C18 column. Detection limits and recoveries were 0.05 µg/ml and 91.8% in serum and 0.5 µg/ml and 101.4% in urine, respectively.

Cefmenoxime. Isocratic methods using a variety of stationary phases were reported for cefmenoxime. A phenyl column was used for cefmenoxime in plasma and urine (54). Urine samples were directly assayed, but plasma samples were deproteinized with acetonitrile before separation and quantitation. Detection limits were 0.2 µg/ml in plasma and 5 µg/ml in urine. A cyan analytical column and a perchloric acid serum deproteinization step formed the basis of another method (57). Urine samples were directly injected. The quantitation limit and recovery were 0.5 µg/ml and 74.6%, respectively. Another serum method used acetonitrile deproteinization, methylene chloride extraction-concentration, and C18 RP analysis to lower the detection limit to 0.62 µg/ml (66). The mobile phase consisted of acetonitrile, 0.05 M ammonium acetate buffer, and 5 mM tetrabutylammonium sulfate. Other sample preparation procedures that have been evaluated include ultrafiltration of plasma samples containing sodium dodecyl sulfate (30), ultrafiltration of plasma and urine samples after the addition of methanol (37), and acetonitrile deproteinization (12).

Cefoperazone. RP-HPLC was used in seven assay methods for cefoperazone in plasma, serum, urine, or CSF. Separation was usually achieved after protein precipitation (23, 35, 60, 65). Urine and CSF samples were either analyzed directly (23, 60) or diluted before analysis (35). One method separated the drug on a phenyl HPLC column after methanol plasma precipitation (49), whereas another method involved an ethyl acetate extraction of acidified samples and separation on a customized stationary phase (20). Several methods used more extensive extraction procedures (3, 17, 24). Cefoperazone was extracted with chloroform-butanol before gradient elution chromatography on a phenyl column (3). Another method used an acidic chloroform-pentanol extraction followed by a neutral back-extraction with separation by RP-HPLC (17). Sep-Pak C18 cartridges were used for sample cleanup before RP-HPLC in a third extraction method (24). A review appeared that advocates LC as an aid to the rational use of antimicrobial agents (73).

Ceftaxime. A report has appeared comparing the results between a bioassay and an LC method for cefotaxime and desacetylcefotaxime analysis in rat serum, human serum, and bile (39). The LC method was more specific than the microbiological method. The LC method was linear from 0.5 to 100 µg/ml for cefotaxime and 1.25 to 100 µg/ml for its desacetyl analog. An RP method for cefotaxime and other cephalosporins in serum gave a detection limit of about 50 ng/ml for serum after extraction (17). In another multi-cephalosporin method, quantitation of the desired cephalosporins at therapeutic concentrations was achieved by judiciously modifying the mobile-phase composition (65).

Recoveries of >95% and detection limits of 0.2 µg/ml were reported. Other generalized cephalosporin methods capable of quantifying cefotaxime utilized Radial-Pak C18 cartridges (21) or gradient elution chromatography (3).

Cefotaxime and its desacetyl metabolite were quantified in serum after trichloroacetic acid deproteinization and separation by C8 RP-HPLC (10). Peak heights at 310 nm were directly proportional to concentration over the range of 0.3 to 40 µg/ml, and results agreed with those of a bioassay method. A similar method used detection at 254 nm (31). A method for cefotaxime, desacetylcefotaxime, and moxalactam in human bile and serum required centrifugation of bile samples and perchloric acid serum deproteinization before
quantitation at 236 nm using RP-HPLC (40). An alternate method for determining serum and urinary cefotaxime and desacyetyl metabolite levels required a chloroform-acetone (1:3) wash to remove proteins and lipids (22). After centrifugation, the aqueous phase was freeze-dried, and then it was reconstituted in mobile phase. Recoveries were 96 ± 11% by RP-HPLC (262 nm). One procedure utilized a chloroform-butanol (3:1) wash for serum, followed by direct injection of the supernatant (76). Urine samples were simply diluted with water and centrifuged. Calibration graphs were rectilinear for 0.5 to 250 and 5 to 500 µg of cefotaxime per ml in plasma and urine, respectively, and for 0.5 to 25 and 5 to 100 µg of desacylcefotaxime per ml, respectively. Precision ranged between 1.1 and 1.8%. A method based on Sep-PAK C₁₈ isolation was examined, and results for cefotaxime compared favorably with those of a microbiological method, but no correlation existed for its metabolite (14). Solid-phase isolation procedures were also used for blood and myometrium samples (8).

Two enzymatic methods based on competitive inhibition of penicillinase by cephalosporin (62) or the colored cephalosporin PADAC (7-[2-thienylacetamido]-3-(4-N,N di-methylaminophenylazo)-pyridinium-methyl)-3-cephem-4-cephalosporidin of penicillinase (45). Quantitation of ceftriaxone, cephalaxin (25), cephaloridine (44), and cefoperazone (73).

Cefpimizole. For cepfimizole analysis, acetonitrile-deproteinized plasma samples and diluted urine samples were filtered before injection onto a C₁₈ column (42). A four-component mobile phase was used: water, methanol, 0.1 M EDTA, and 0.4 M tetrabutylammonium hydroxide (251:140:34:5). Quantitation limits were 0.33 µg/ml in plasma and 16.8 µg/ml in urine, with corresponding recoveries of 78.7 and 99.3%.

Cefsulodin. Two LC methods were reported during this review period for quantifying cefsulodin and other cephalosporins after deproteinization and separation by RP-HPLC (37, 44). An LC method reported for the simultaneous determination of cefotiam and cefslodin in serum and bone marrow was described in the cefotiam section (75). Plasma samples were buffered to prevent degradation, centrifuged, and directly injected into an RP-HPLC system with detection at 254 nm (29). The intra- and interassay precisions were ca. 2 and 3 to 4%, respectively. Calibration curves were linear from 0.2 to 200 µg/ml. In a similar method, plasma samples were mixed with an equal volume of pH 6 phosphate buffer, and proteins were precipitated using cold methanol (1). The mixtures were stored in an ice bath to prevent degradation. Urine samples were initially diluted with buffer. A flow gradient from 0.3 to 2.0 ml/min over 34 min was required to achieve acceptable selectivity using RP-HPLC. A procedure requiring plasma precipitation or direct injection of CSF was previously reported (48). This method was linear from 0.5 to 8 and 1 to 100 µg of cefsulodin per ml in CSF and plasma, respectively.

Ceftazidime. An RP method appeared for quantifying serum and urinary ceftazidime (45). Serum samples were deproteinized with methanol, and urine samples were assayed directly using RP-HPLC, with detection at 255 nm. Calibration curves were linear from 1.9 to 30 µg/ml. Recoveries were greater than 90% at 3, 15, and 30 µg of ceftazidime per ml, and the method was specific for the drug in the presence of 11 other β-lactams. A similar method was capable of analyzing plasma, urine, CSF, and peritoneal dialysis samples (34). Other methods utilized a Hypersil ODS column (5) or a Micropak MCH column (50) for effecting the selective determination of ceftazidime in serum or urine.

Ceftizoxime. A method for quantifying ceftizoxime in rat serum, bile, and urine used acetonitrile deproteinization (67). The drug in the filtered supernatant was selectively quantified on a µBondapak alkyl-phenyl column with detection at 280 nm (bile and urine) and 254 nm (serum). Ceftizoxime was stable in solution for at least 6 h at room temperature and for 2 days at 4°C. The method was linear between 0.2 and 5 µg/ml (serum), 10 and 500 µg/ml (bile), and 100 and 4,000 µg/ml (urine). A modified version has also appeared (43) which utilizes ceftazidime as an internal standard. Recently, a method appeared for quantifying drug in normal and uremic patient sera (46). Similar sample pretreatment procedures and chromatographic conditions were used, but 310 nm was chosen for quantitation. This method was linear between 1.5 and 100 µg/ml and correlated well with a bioassay (r = 0.9832). A DEAE Sephadex A-25 anion-exchange column formed the basis of another selective cepfizoxime method (26). Serum samples were applied directly to the column and washed with a pH 7.2 phosphate-saline solution. After elution (1 M NaCl), 0.1 ml of the processed sample was injected into an RP-HPLC system, and separation was achieved with an isocratic acetonitrile-acetic acid (13:87) mobile phase. A systematic approach for cephalosporin analysis in human plasma, urine, bile, and milk was proposed (59). This approach investigated the effects of pH and ion strength and concluded that cephalosporins are strongly retained on octadecylsilyl stationary phases with the use of low-ion-strength acidic mobile phases. A cepfizoxime method was presented that is capable of measuring the drug in plasma and urine at concentrations as low as 0.2 µg/ml in plasma and 25 µg/ml in urine.

Ceftriaxone. An ion-pairing (hexadecyltrimethylammonium) RP-HPLC method was reported for analyzing ceftriaxone in plasma, urine, and bile (71). Plasma samples were deproteinized with ethanol and centrifuged before a supernatant sample was injected into an RP-HPLC system. The drug was isolated from urine or bile samples, using acetonitrile, phosphate buffer, and either tetracetylamineum or tetraoctylammonium as pairing agent, before injection. Detection limits were 0.5 µg/ml in plasma and 5 µg/ml in urine and bile. Another method for ceftriaxone in plasma, urine, and saliva used acetonitrile deproteinization followed by analysis on a LiChrosorb Amino column with an isocratic mobile phase consisting of acetonitrile–water–10% ammonium carbonate (35:13:2) with quantitation at 274 nm (4). An RP method has appeared that is applicable to ceftriaxone serum, urinary, and CSF determinations (13). After acetonitrile deproteinization, samples were washed with dichloromethane, and then they were quantified at 280 nm. The method was rectilinear from 5 to 400 µg/ml in serum and urine and from 0.5 to 50 µg/ml in CSF. Serum and pelvic tissue concentrations of ceftriaxone can be determined by a method described above for cefazolin (6).

**SUMMARY**

Since 1980, RP chromatography has been the principal analytical technique used for cephalosporins. This technology offers selectivity, accuracy, and ease of use. Most of the methods rely on protein precipitation and, to a lesser extent, solid-phase isolation or extraction procedures.

The proper selection of a method depends on the analytical constraints imposed by the overall objective of the
study. For example, pharmacokinetic datum interpretation mandates that the method be validated and provide specific and accurate results. LC is the preferred technique, since it not only meets these specifications but may also distinguish between the drug and metabolites. Those chromatographic methods which quantify several different cephalosporins are not desirable for pharmacokinetic datum interpretation, since accuracy and precision are usually compromised in order that many different drugs may be quantified in a single analysis. The proper selection of sample preparation methods is dependent on the presence of potential interferences and the acceptable lower limit of quantitation. Protein precipitation methods offer ease of sample preparation but may suffer from nonselectivity. Solid-phase isolation and extraction procedures may increase selectivity and improve the limit of quantitation. Although LC provides specific and accurate results, clinical laboratories may prefer to use the less specific methods for therapeutic drug monitoring. In this case, microbiological, enzymatic, and fluorimetric methods offer improved sample throughput but less specificity. However, these methods should not be used for drugs that may have a low margin of safety or if the patient is on multiple-antibiotic therapy.

Future methods may involve incorporating solid-phase isolation columns to enhance the specificity of chromatographic, microbiological, enzymatic, and fluorescence methods. Advancements in microbore column technology may allow improvements in the selectivity and sensitivity of LC methods. Many investigators prefer to use simple protein precipitation procedures for sample preparation because of sample throughput constraints. However, advances in robotic sample preparation may allow the more cumbersome solid-phase isolation or extraction techniques to be used to improve sample throughput and specificity.

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LITERATURE CITED


