RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2010.36.297

ScienceAsia 36 (2010): **297–304**

Development and validation of high performance liquid chromatographic method for the simultaneous determination of ceftriaxone and vancomycin in pharmaceutical formulations and biological samples

Abu Tariq, Masoom Raza Siddiqui, Jitendra Kumar, Dinesh Reddy, Prithvi Singh Negi, Manu Chaudhary, Sanjay Mohan Srivastava, Raj Kumar Singh*

Venus Medicine Research Centre, Hill Top Industrial Estate, EPIP Phase I, Jharmajri (Extn.) Bhatoli Kalan, Baddi, Solan (H.P.) India-173205

*Corresponding author, e-mail: drrksingh@venusremedies.com

Received 8 Sep 2009
Accepted 4 Aug 2010

ABSTRACT: A reverse phase-liquid chromatographic method with UV detection at 280 nm is described for simultaneous determination of ceftriaxone sodium and vancomycin hydrochloride. Chromatographic separation of the two drugs was achieved on a Betasil C-1 column using a mobile phase consisting of a binary mixture of acetonitrile and triethylamine buffer adjusted to pH 3.5 ± 0.1 with orthophosphoric acid in a ratio of 20:80. The liquid chromatographic method developed offers symmetric peak shape, good resolution, and reasonable retention time for both drugs. Linearity, accuracy, and precision were found to be acceptable over the concentration ranges 125–750 ppm for ceftriaxone and 62.5–375 ppm for vancomycin. The liquid chromatographic method was successfully applied to the quality control of formulated products, plasma, and cerebrospinal fluid samples containing ceftriaxone and vancomycin.

KEYWORDS: liquid chromatography, ceftriaxone sodium, vancomycin hydrochloride, triethylamine, cerebrospinal fluid, quality control

INTRODUCTION

Ceftriaxone is (6*R*,7*R*) - 3 [(acetyl - oxy) methyl] - 7 - [[(2*Z*)-(2-amino-4-thiazolyl)(methoxyamino)-acetyl] amino] - 8 - oxo - 5 - thia - 1 - azabicyclo[4.2.0]oct - 2 - ene - 2 - carboxylic acid [1]. Ceftriaxone is a cephalosporin beta-lactam antibiotic used in the treatment of bacterial infections caused by susceptible, usually gram positive microorganisms. The bactericidal activity of ceftriaxone results from the inhibition of the cell wall synthesis and is mediated by ceftriaxone binding to penicillin-binding proteins. It inhibits the mucopeptide synthesis in the bacterial cell wall. The beta-lactam moiety of ceftriaxone binds to carboxypeptidase, endopeptidase, and transpeptidase in the bacterial cytoplasmic membrane. These enzymes are involved in cell wall synthesis and cell division. By binding to these enzymes, ceftriaxone results in the formation of defective cell walls and cell death. Vancomycin hydrochloride is a glycopeptide antibiotic which can be isolated from both *Streotomyces orientalis* and *Nocardia lurida* [2]. It inhibits bacterial cell wall synthesis. Vancomycin prevents the synthesis of peptidoglycan by binding tightly to peptides containing D-alanyl-D-alanine at the free carboxyl end. Vancomycin has also been shown to alter the permeability of the cell membrane and may selectively inhibit RNA synthesis. Introduced in 1956, vancomycin has strong bactericidal activities against many gram positive bacteria, particularly *Staphylococcus aureus*. Due to its toxicity vancomycin was relegated to the role of alternate therapy when antibiotics such as methicillin became available [3].

There are several methods to estimate ceftriaxone, including high performance liquid chromatography (HPLC) [4–8], high performance thin layer chromatography [9], capillary electrophoresis [10, 11], and spectrophotometry [12–14]. In case of vancomycin, it has been assayed by HPLC [15–17], micellar electrokinetic capillary chromatography [18], and spectrophotometry [19].

Immunox-V (ceftriaxone-vancomycin) is a synergetic antimicrobial combination with marked in vitro antibacterial activities against broad spectrum gram-negative and gram-positive bacteria. Since ceftriaxone is less active against gram-positive cocci than first generation cephalosporins, marked activities are seen

against gram-negative bacteria such as Enterobacteriaceae including beta-lactamase producing strains and penicillin resistant strains such as *Haemophilus influenzae*, *Neisseria gonorrhea*, and *N. meningitidis*. It is active against *S. aureus* but not against methicillin-resistant *S. aureus*. However vancomycin acts against a variety of gram-positive bacteria including MRSA and *S. epidermidis*. By combining these two drugs a fixed dose combination developed covers wide range of gram-positive and gram-negative bacteria. The combination is made compatible by adding a chemical vector which further reduces toxic effects.

The synergistic action of Immunox-V has strong bactericidal activity against *S. aureus* and methicillin-resistant strains. The combination works well against enterococci, prevents development of resistance, and has been used successfully against a wide range of bacterial infections including bacterial meningitis.

The present communication describes an isocratic liquid chromatography (LC) method for simultaneous determination of ceftriaxone sodium and vancomycin hydrochloride, which would be used for the quality control of the formulation developed and other biological applications. The advantage of this method is that by doing one column analysis one can save time and resources. To the best of our knowledge there is at present no method to determine the combined dosage form simultaneously. This study achieved satisfactory results in terms of selectivity, linearity, precision, and accuracy under simple chromatographic conditions.

MATERIALS AND METHODS

Chemicals and reagents

Ceftriaxone sodium and vancomycin hydrochloride reference standards (RS) of US Pharmacopoeia were bought from Sigma, US. Immunox-V, a fixed dose combination was obtained from the manufacturer (Venus Remedies Limited, India). Each vial contained 1 g ceftriaxone and 0.5 g vancomycin. Triethylamine (TEA), acetonitrile, and phosphoric acid were of chromatographic grade and were obtained from Merck, Germany. All other chemicals were of analytical reagent grade unless specified.

Chromatographic separation was performed on an Agilent 1200 series liquid chromatographic system equipped with G1311A quaternary pump, Agilent variable-wavelength UV-Vis detector, and a G1329A auto injector. EZ CHROME ELITE software was employed for data collecting and processing. Samples were centrifuged by using a Remi Centrifuge (C-24BL).

Chromatographic conditions

Chromatographic separation was performed on a Betasil C-1 stainless steel column with dimensions 250 mm $\times$ 4.6 mm, 5 µm (Thermo Electron Corporation). The mobile phase consisting of a binary mixture of acetonitrile and TEA buffer adjusted to pH 3.5 ± 0.1 with orthophosphoric acid in a ratio of 20:80, was delivered at rate of 1.0 ml/min. The mobile phase was filtered through 0.45 µm membrane nylon filter (Millipore) and degassed prior to use. Separation was performed at 40 °C, with an injection volume of 10 µl run for 10 min, and detected at 280 nm.

Preparation of buffer solution

TEA (2 ml) was dissolved in 998 ml of milli-Q water and adjusted to pH 3.5 ± 0.1 with dilute orthophosphoric acid. The preparation was then filtered through 0.22 µm nylon filter (Millipore) and degassed. For the preparation of the standard and sample solutions, the mobile phase was used as the diluent.

Preparation of Solutions

A standard solution was prepared by dissolving accurately weighed quantities of ceftriaxone sodium (RS) 50 mg and vancomycin hydrochloride (RS) 25 mg in the mobile phase, and diluting quantitatively and stepwise with mobile phase to obtain a solution of a known concentration of about 500 ppm of ceftriaxone sodium and 250 ppm vancomycin hydrochloride.

For the preparation of sample solution, accurately weighed (75 mg) Immunox-V, (ceftriaxone sodium and vancomycin hydrochloride for injection), was transferred to a 100-ml volumetric flask. The mobile phase was added, then swirled to dissolve it, and then diluted to a volume of 100 ml with the mobile phase.

Preparation of plasma and cerebrospinal spinal fluid (CSF) sample

Rats (weighing 150–200 g) were randomly selected for carrying out the study. They were housed at controlled temperature and humidity in an alternating 12-h light and dark cycle with free access to food and water. The study was approved by the institutional animal ethical committee.

The drugs, 500 µg/ml of ceftriaxone and 250 µg/ml of vancomycin, were given to each animal according to their body weight, intravenously for 7 days. After day 7, samples were collected for analysis.

Blood samples were collected in 3.8% sodium citrate containing vials for plasma preparation. Blood-citrate containing tubes were centrifuged at 2000g for

15 min at ambient temperature and the supernatant was collected very carefully. Then, 1 ml plasma was mixed with an equal amount of chilled acetonitrile solution containing water and acetonitrile in a 50:50 ratio and kept for 10 min to precipitate proteins. Samples were centrifuged again at 2500g for 20 min, the supernatant was aspirated out, and an aliquot of 10 µl was injected into the HPLC system. The supernatants were diluted with diluent if required for the analysis of drug assay in sample.

Preparation of CSF samples

Cerebrospinal fluid (CSF, 20–40 µl) was obtained by puncture of the cisterna magna. The sample was mixed with 100 µl of acetonitrile and kept for 10 min to precipitate the plasma proteins. Each samples was centrifuged at 2000g for 20 min at 0–4 °C and supernatants were aspirated out carefully for analysis of antibiotic drug concentration separately.

Data analysis

To determine ceftriaxone sodium and vancomycin hydrochloride separately, we injected equal volumes of the standard preparation and the assay preparation into the chromatograph, recorded the chromatograms, and measured the responses for the major peaks.

RESULTS AND DISCUSSION

Method development and validation

Several methods are available to analyse ceftriaxone and vancomycin individually, but no such method is available for the simultaneous determination of these two drugs. A literature survey reveals the analysis of ceftriaxone using spectrophotometry[20] and Folin-Ciocalteu reagent in the presence of 20% sodium carbonate solution. The blue colour from the chromogen formed was measured at wavelength of maximum absorption of 750 nm for ceftriaxone against reagent blank. For ceftriaxone the chromogen was linear over the range of 2.0–36 µg/ml. The separation and identification of vancomycin was done by thin-layer chromatography, normal and reverse phases chromatography[21–24].

Thus we developed a single method for the combined analysis of both drugs, saving time and other resources. Taking into consideration the instability of ceftriaxone sodium and vancomycin hydrochloride in strong alkaline and strong acidic conditions, the pH value of the mobile phase should be limited within the range of 3–5, since mild acidic pH favours the retention and separation of two drugs on a Betasil C-1 column. After some trials, the TEA buffer with pH 3.5 ± 0.1 was finally selected. Acetonitrile is the most commonly used solvent for LC analysis and often is the first choice for many researchers. Therefore, a binary mixture of acetonitrile and TEA buffer became the initial mobile phase for the determination of the two drugs. Firstly, various concentrations of TEA buffer were tried in order to achieve the proper conditions. We found that 0.014 M TEA buffer was ideal for our work. Then, the proportion of acetonitrile and TEA buffer in mobile phase was determined by varying the proportion of acetonitrile and TEA buffer from 20:80, 25:75 to 30:70.

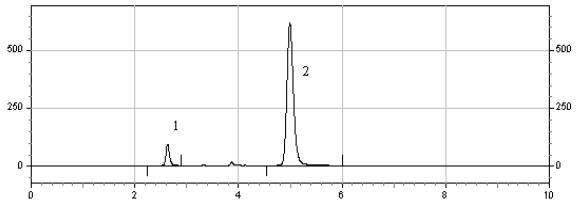

Fig. 1 A standard LC chromatogram of vancomycin (250 ppm, retention time 2.6 min, peak 1) and ceftriaxone (500 ppm, retention time 4.8 min, peak 2).

Since both ceftriaxone sodium and vancomycin hydrochloride in the mobile phase have no significant UV maximum, 280 nm was employed for the detection to ensure the sensitivity of the method.

After a number of preliminary experiments, a Betasil C-1 column with a TEA buffer in a 20:80 ratio with acetonitrile was selected for the determination of the two drugs. This system produced symmetric peak shape, good resolution, and reasonable retention time for both the drugs. The average retention times of ceftriaxone sodium and vancomycin hydrochloride for 6 repetitions were 4.8 ± 0.08 and 2.6 ± 1.05 min, respectively (Fig. 1).

Both drugs were forced to degrade under several stress conditions and it was found that both drugs show poor stability under acidic, basic, and oxidative conditions. In the case of thermal- and photo-degradation the degradation was found to be 70% in 24 h.

System suitability experiments gave satisfactory results (Table 1). It was found that the relative standard deviation (RSD) for both drugs was less than 2%.

Under optimized experimental conditions a straight-line calibration curve was obtained for the concentration ranges of 125–750 ppm for ceftriaxone and 62.5–375 ppm for vancomycin. Linear regression analysis using the method of least squares treatment of calibration data ($n = 6$) was made to evaluate

Table 1 System performance parameters for ceftriaxone sodium and vancomycin hydrochloride ($n = 5$).

Peak	Compounds	t_R (min)	N	K	Res	α
1	Ceftriaxone	4.8	10 816	470.66	—	—
2	Vancomycin	2.6	7226	257.33	14.08	0.55

t_R = retention time, N = theoretical plates, K = capacity factor, Res = resolution, α = selectivity factor.

slope, intercept, and correlation coefficient. The linear regression equation was $A = 56\,274\,C - 43\,293$ for ceftriaxone sodium and $A = 4444.09\,C + 2791.91$ for vancomycin hydrochloride, where A is the absorbance and C is the concentration of the drug, with a correlation coefficient $r = 0.99992$ for ceftriaxone and $r = 0.99985$ for vancomycin.

The limit of detection (LOD) was calculated and found to be 0.10 ppm for ceftriaxone and 0.18 ppm for vancomycin and limit of quantification (LOQ) was found to be 0.30 ppm for ceftriaxone and 0.56 ppm for vancomycin.

The accuracy of the proposed method was checked by recovery experiment. Mean percentage recoveries from the combination formulation was found to be 100.2 ± 0.56 for ceftriaxone and 101.48 ± 0.55 for vancomycin indicating good accuracy of the method for simultaneous determination of the two drugs. Acceptable precision was achieved for ceftriaxone and vancomycin (Table 2), as revealed by the relative standard deviation data (RSD $<$ 2.0% in all levels of the two drugs).

The solution stability of the two drugs was monitored by keeping the solutions at room temperature under darkness and in the refrigerator. The results show no significant difference in the area after 6 h. The cumulative RSD at room temperature and in the refrigerator were found to be 1.60% and 1.40% for ceftriaxone, 1.41% and 1.50% for vancomycin, respectively.

The application of the method was checked by analysing the plasma and cerebrospinal fluid samples. The lower limit of quantification was found to be 1.5 µg/ml for ceftriaxone and 3.2 µg/ml for vancomycin, which shows the efficiency of the developed method for plasma analysis. The inter-day precision of the method was, on average, 4% or better over the same concentration range, with an assay recovery of 95% for ceftriaxone and 94% for vancomycin, respectively. The same method was applied for the analysis of CSF samples obtained, and the method shows good detection of drugs, i.e., 8.7 µg/ml of ceftriaxone and 6.73 µg/ml of vancomycin.

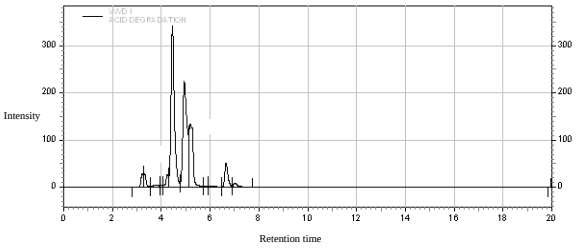

Fig. 2 A LC chromatogram of acid degradation of ceftriaxone and vancomycin.

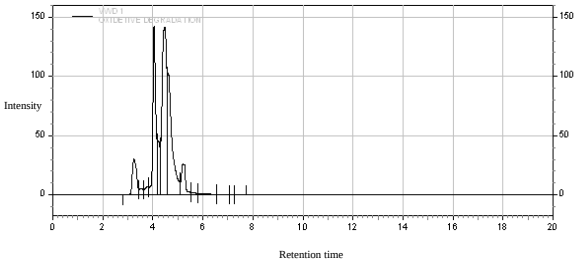

Fig. 3 A LC chromatogram of oxidative degradation of ceftriaxone and vancomycin.

Selectivity

Selectivity is the ability of an analytical method to differentiate and quantify the analyte in the presence of other components in the sample. It can be determined by analysing forced degraded powder samples. Forced degradation studies were performed to provide an indication of the stability of the proposed method by exposing the formulation product to the stress conditions of UV light, high temperature (105 °C), acid (0.5 M HCl), and base (0.5 M NaOH) in order to test the ability of the proposed method to separate the active component. The samples were degraded to levels where the contents of ceftriaxone and vancomycin in the samples were lowered to that of the original level. Chromatograms for the acid and oxidative degradation are shown in Figs. 2 and 3. Under the given stress conditions both drugs are unstable and significantly degraded peaks appear. RRT was calculated. In the case of forced degradation by an oxidant, the relative retention time was calculated to be 3.16, 3.51, 3.80, 3.95, 4.15, 4.48, 4.85, 5.28, 5.60, 6.01, 6.85, 7.10, 7.50. When the samples were subjected to acid degradation the relative retention time was found to be 3.1, 3.18, 3.71, 4.06, 4.18, 4.48, 4.81, 5.15, 5.80, 6.13, 6.80, 7.06.

Table 2 Precision of the HPLC method for simultaneous determination of ceftriaxone sodium and vancomycin hydrochloride.

	Method Precision		Intermediate Precision	
Drug	Mean ± SD	RSD (%, $n = 6$)	Mean ± SD	RSD (%, $n = 2$)
Ceftriaxone	100.84 ± 0.41	0.40	100.29 ± 0.18	0.18
Vancomycin	101.91 ± 0.66	0.64	101.36 ± 0.35	0.34

Specificity

Specificity is the ability of the method to accurately measure the analyte in the presence of all potential sample components. The analyte peak is evaluated for peak purity from the nearest eluting peak. For this purpose a solution containing 500 ppm of ceftriaxone sodium and 250 ppm of vancomycin hydrochloride was injected and peak purity was monitored. The acceptance criteria for peak purity is that the purity angle should be less than the purity threshold. The peak purity was found to be 0.999.

To check the specificity, peak purity was determined. Peak purity analysis for ceftriaxone and vancomycin gave a total purity angle of 0.99 and 1.0, respectively, which was considered to be satisfactory.

System suitability

System performance parameters of the developed HPLC method were determined by analysing standard working solutions. Chromatographic parameters, such as number of theoretical plates, resolution, capacity factor, and selectivity factor were determined (Table 1). The results indicate the good performance of the system. System repeatability was determined by 5 replicate injections of a working standard solution, and the relative standard deviations of peak areas of both drugs were calculated to evaluate the repeatability.

Linearity

Linearity of calibration curves for both ceftriaxone sodium and vancomycin hydrochloride for 6 concentration levels each was found to be satisfactory. Peak area and concentration of each drug substance was subjected to regression analysis to calculate the regression equation and the correlation coefficients. The results show that within the tested concentration range there was a strong correlation between the peak area and the concentration of each drugs. The system was found to be linear in range of 125–750 ppm for ceftriaxone and 62.5–375 ppm for vancomycin.

Limit of detection and limit of quantitation

Limit of detection (LOD) was established at a signal to noise ratio (S/N) of 3.3. Limit of quantification (LOQ) was established at a signal to noise ratio (S/N) of 10. LOD and LOQ were experimentally verified by 6 injections of ceftriaxone sodium and vancomycin hydrochloride at the LOD and LOQ concentration.

Accuracy

Accuracy was determined by applying the described method to the known amount of each drug corresponding to 70, 100, and 130% of test solution. The accuracy was then calculated as the percentage of analyte recovered by the recovery study. The recovery of ceftriaxone and vancomycin in test samples ranged from 80.0 to 120.0%.

Robustness

Robustness, as documented in the ICH guidelines[25], should be considered early in the development of a method. If the results are susceptible to variations in method conditions, these conditions must be adequately controlled. The effect of variations in some experimental conditions was tested. In all the deliberately varied chromatographic conditions such as flow rate, mobile phase variation, pH, temperature variation, and column, the results were found to be satisfactory for ceftriaxone and vancomycin. Thus the operational conditions for the proposed method were considered to be very robust.

Analytical solution stability

The analytical solution stability of both the standard and the test was determined by monitoring the peak area responses of the standard solution and a sample solution of ceftriaxone sodium and vancomycin hydrochloride at 0, 2, 4, and 6 h at room temperature and in the refrigerator.

Method application

The validated LC method was applied to the simultaneous determination of ceftriaxone and vancomycin for injection. The three batches of the sample were

Table 3 Assay results for ceftriaxone sodium and vancomycin hydrochloride sterile powder for injection (mean ± SD).

Batch No.	Ceftriaxone sodium % ($n = 3$)	Vancomycin hydrochloride % ($n = 3$)
1	100.42 ± 0.21	100.11 ± 0.24
2	99.98 ± 0.17	100.05 ± 0.12
3	100.24 ± 0.15	99.23 ± 0.18

analysed and the assay results, expressed as percentage of the label claim, are shown in Table 3. The results indicate that the amount of each drug in the injection corresponds to the requirement.

Clinical application

In pharmaceutical industries, the use of HPLC has been associated with antibiotics analysis and this technology has become available for quality control. In the clinical laboratory the use of this technique has been efficient for determination of analytes in several biological fluids. Therefore, HPLC has been developed for the analysis of vancomycin in medicines, as well as for the determination in biological fluids [17, 24, 26–34].

Kitahashi and Furuta[35] developed an assay for measuring vancomycin concentration by micellar electrokinetic capillary chromatography with detection at 210 nm using direct serum injection. The migration time of vancomycin was about 7 min. The linearity was from 0 to 100 μg/ml and the limit of detection was 1.0 μg/ml. No observed interference from 32 other antibiotics was observed. Shibata et al[36] described a novel HPLC tandem mass spectrometry method for determination of vancomycin in serum and urine without treatment. Khasanov et al[37] determined ceftriaxone in blood samples and tissues using ion-exchange chromatography. Several other studies show the analysis of ceftriaxone in biological samples [38, 39].

We successfully applied the current method for the analysis of the plasma samples obtained. The plasma samples were assayed for ceftriaxone and vancomycin by an automated, reversed phase HPLC procedure mentioned above. The lower limit of quantification of the result established at a signal to noise ratio (S/N) of 10 was determined. A typical LC chromatogram showing the detection of ceftriaxone and vancomycin in plasma samples is shown in Fig. 4. These results show that our method can be used for the simultaneous determination of ceftriaxone sodium and vancomycin hydrochloride in the pharmaceutical companies and research laboratories for routine and biological samples analysis.

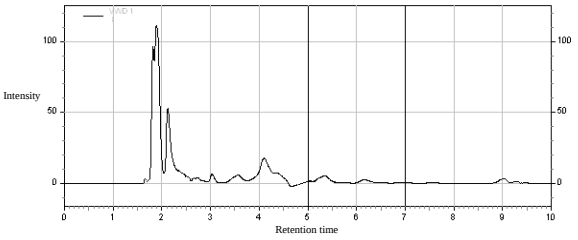

Fig. 4 A typical LC chromatogram of blank plasma.

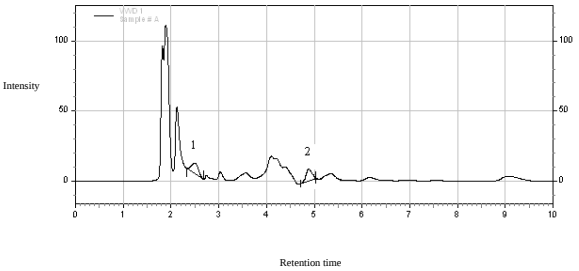

Fig. 5 A typical LC chromatogram of plasma sample.

Acknowledgements: The authors are grateful to Chief General Manager, Domestic Operations, Venus Remedies Limited for providing samples of Immunox-V, Venus Medicine Research Centre for providing funds and other resources for carrying out research. M.R. Siddiqui is thankful to Dr Nafisur Rahman, Dept. of Chemistry, Aligarh Muslim University for his valuable suggestions.

REFERENCES

1. Budavari S (2001) *The Merck Index*, 13th edn, Merck & Co., Whitehouse station, NJ.
2. Perkins HR (1982) Vancomycin and related antibiotics. *Pharmacol Therapeut* **16**, 181–97.
3. Cafferkey MT, Hone R, Kaene CT (1982) Severe staphylococcal infections treated with vancomycin. *J Antimicrob Chemother* **9**, 69–74.
4. Marini D, Balestrieri F (1986) Determination of ceftriaxone by HPLC. *Il Farmaco* **41**, 172–6.
5. Bowman DB, Aravind MK, Miceli JN, Kauffman RE (1984) Reversed-phase high-performance liquid chromatographic method to determine ceftriaxone in biological fluids. *J Chrom B* **309**, 209–13.
6. Ascalone V, Dal Bol L (1983) Determination of ceftriaxone, a novel cephalosporin, in plasma, urine and saliva by high-performance liquid chromatography on an NH_2 bonded-phase column. *J Chrom B* **273**, 357–66.
7. Jungbluth GL, Jusko WJ (1989) Ion-paired reversed-phase high-performance liquid chromatography assay for determination of ceftriaxone in human plasma and urine. *J Pharmaceut Sci* **78**, 968–70.
8. Joshi S (2002) HPLC separation of antibiotics present

in formulated and unformulated samples. *J Pharmaceut Biomed Anal* **28**, 795–809.
9. Eric-Jovanovic S, Agbaba D, Zivanov-Stakic D, Vladimirov S (1998) HPTLC determination of ceftriaxone, cefixime and cefotaxime in dosage forms. *J Pharmaceut Biomed Anal* **18**, 893–8.
10. Pajchel G, Owski KP, Tyski S (2002) CE versus LC for simultaneous determination of amoxicillin/clavulanic acid and ampicillin/sulbactam in pharmaceutical formulations for injections. *J Pharmaceut Biomed Anal* **29**, 75–81.
11. Zhang D, Yongjun MA, Zhou M, Li L, Chen H (2006) Determination of ceftriaxone sodium in pharmaceutical formulations by flow injection analysis with acid potassium permanganate chemiluminescence detection. *Anal Sci* **22**, 183–6.
12. Zhao W, Zhang Y, Li Q (2008) Indirect spectrophotometric determination of sodium ceftriaxone with *n*-propyl alcohol-ammonium sulfate-water system by extraction flotation of copper(II). *Clin Chim Acta* **391**, 80–4.
13. Granich GG, Krogstad DJ (1987) Ion pair high-performance liquid chromatographic assay for ceftriaxone. *Antimicrob Agents Chemother* **31**, 385–8.
14. Al-Momani IF (2001) Spectrophotometric determination of selected cephalosporins in drug formulations using flow injection analysis. *J Pharmaceut Biomed Anal* **25**, 751–7.
15. de Jesús Valle MJ, González López F, Sánchez Navarro A (2008) Development and validation of an HPLC method for vancomycin and its application to a pharmacokinetic study. *J Pharmaceut Biomed Anal* **48**, 835–9.
16. Forlay-Frick P, Nagy ZB, Fekete J, Kettrup A, Gebefugi I (2001) Reverse phase HPLC method for determination of vancomycin in influlenza vaccine. *J Liq Chrom Relat Tech* **24**, 497–507.
17. Farin D, Piva GA, Gozlan I, Kitzes-Cohen R (1998) A modified HPLC method for the determination of vancomycin in plasma and tissues and comparison to FPIA (TDX). *J Pharmaceut Biomed Anal* **18**, 367–72.
18. Jing-wu K, Schepdael AV, Roets E, Hoogmartenis J (2001) Analysis of vancomycin and related impurities by micellar electrokinetic capillary chromatography. Method development and validation. *Electrophoresis* **22**, 2588–92.
19. Rodrigues A Jr, Vila MMDC, Tubino M (2008) Green spectrophotometric method for the quantitative analysis of vancomycin in pharmaceuticals and comparison with HPLC. *Anal Lett* **41**, 822–36.
20. Patel SA, Patel NM, Patel MM (2006) Spectrophotometric estimation of cefotaxime and ceftriaxone in pharmaceutical dosage forms. *Indian J Pharmaceut Sci* **68**, 101–3.
21. Rao GR, Kanjilal G, Mohan KR (1978) Extended application of Folin-Ciocalteu reagent in the determination of drugs. *Analyst* **103**, 993–4.
22. Hu MW, Anne L, Forni T, Gottwald K (1990) Measurement of vancomycin in renally impaired patient samples using a new high-performance liquid chromatography method with vitamin B12 internal standard: comparison of high-performance liquid chromatography, emit, and fluorescence polarization immunoassay methods. *Ther Drug Monit* **12**, 526–9.
23. Kirchmeier RL, Upton RP (1978) Simultaneous determination of vancomycin, anisomycin, and trimethoprim lactate by high pressure liquid chromatography. *Anal Chem* **50**, 349–51.
24. Diana J, Visky D, Roets E, Hoogmartens J (2003) Development and validation of an improved method for the analysis of vancomycin by liquid chromatography: Selectivity of reversed-phase columns towards vancomycin components. *J Chrom A* **996**, 115–31.
25. ICHQ2B (1997) Text on validation of analytical procedures methodology. In: International Conference on Harmonization of Technical Requirements for the Registration of Drugs For Human Use, Geneva, Switzerland, March 1979. Published in Federal Register, vol 62, pp 27463–7.
26. Vila MMDC, Oliveira RM, Gonçalves MM, Tubino M (2007) Analytical methods for vancomycin determination in biological fluids and in pharmaceuticals. *Química Nova* **30**, 395–9.
27. Jehl F, Gallion C, Thierry RC, Monteil H (1985) Determination of vancomycin in human serum by high-pressure liquid chromatography. *Antimicrob Agents Chemother* **27**, 503–7.
28. Demotes-Mainard F, Labat L, Vinçon G, Bainnwarth B (1994) Column-switching high-performance liquid chromatographic determination of vancomycin in serum. *Ther Drug Monit* **16**, 293–7.
29. Li L, Miles MV, Hall W, Carson S (1995) An improved micromethod for vancomycin determination by high-performance liquid chromatography. *Ther Drug Monit* **17**, 366–70.
30. del Nozal MJ, Bernal JL, Pampliega A, Marinero P, López MI, Coco R (1996) High-performance liquid chromatographic determination of vancomycin in rabbit serum, vitreous and aqueous humour after intravitreal injection of the drug. *J Chrom A* **727**, 231–8.
31. Saito M, Santa T, Tsunoda M, Hamamoto H, Usui N (2004) An automated analyzer for vancomycin in plasma samples by column-switching high-performance liquid chromatography with UV detection. *Biomed Chrom* **18**, 735–8.
32. Cass TR, Villa JS, Karr DE, Schmidt DE Jr (2001) Rapid bioanalysis of vancomycin in serum and urine by high-performance liquid chromatography tandem mass spectrometry using on-line sample extraction and parallel analytical columns. *Rapid Comm Mass Spectrom* **15**, 406–12.
33. Plock N, Buerger C, Kloft C (2005) Successful management of discovered pH dependence in vancomycin recovery studies: novel HPLC method for microdialysis and plasma samples. *Biomed Chrom* **19**, 237–44.

34. Vera López KJ, Bettoluci DF, Vicente KM, Dell'Aquilla AM, Jorge Santos SRC (2007) Simultaneous determination of cefepime, vancomycin and imipenem in human plasma of burn patients by high performance liquid chromatography. *J Chrom B* **860**, 241–5.
35. Kitahashi T, Furuta I (2001) Determination of vancomycin in human serum by micellar electrokinetic capillary chromatography with direct sample injection. *Clin Chim Acta* **312**, 221–5.
36. Shibata N, Ishida M, Prasad YVR, Gao W, Yoshikawa Y, Takada K (2003) Highly sensitive quantification of vancomycin in plasma samples using liquid chromatography-tandem mass spectrometry and oral bioavailability in rats. *J Chrom B* **789**, 211–8.
37. Khasanov VV, Sokolovich EG, Dychko KA (2006) Determination of ceftriaxone in blood and tissues using ion-exchange chromatography. *Pharmaceut Chem J* **40**, 109–11.
38. Hakim L, Bourne DWA, Triggs EJ (1988) High performance liquid chromatographic assay of cefotaxime, desacetylcefotaxime and ceftriaxone in rat plasma. *J Chrom B* **424**, 111–7.
39. Soback S, Ziv G (1988) Pharmacokinetics and bioavailability of ceftriaxone administered intravenously and intramuscularly to calves. *Am J Vet Res* **49**, 535–8.