INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Pharmaceutical Sciences

Research Article……!!!

Received; accepted

TO FORMULATE THE FIXED DOSE COMBINATION OF CEFEPIME AND AMIKACIN

Prabh Simran Singh[1]*, Gagan Shah[2], Rajiv Bharti[3]

1. S.B.S College of Pharmacy, Patti, Taran- Tarn 143416, Punjab, India.
2. B.I.S College of Pharmacy, Moga, Punjab. India.
3. Lala Laj Pat Rai College of Pharmacy, Moga, Punjab. India.

Keywords:

Glomerular filtration, Cefepime, Aminoglycosides

For Correspondence:

Prabh Simran Singh

S.B.S College of Pharmacy, Patti, Taran-Tarn 143416 Punjab, India.

E-mail:

prabh.7750@gmail.com

ABSTRACT

Pharmacokinetic interactions between beta-lactam and aminoglycoside antibiotics have been studied extensively. Concurrent administration of cefepime and amikacin did not alter the pharmacokinetics of either antibiotic. The values reported for $t_{1/2}$, CL, CLR, and Vd, for these two antibiotics are very similar. Like most other cephalosporins and aminoglycosides, cefepime and amikacin are minimally metabolized in humans and are primarily excreted in urine in unchanged forms. The CLR values of cefepime and amikacin indicate that these antibiotics are primarily excreted by glomerular filtration. Since cefepime and amikacin are not significantly metabolized, are excreted primarily in urine by the glomerular filtration process, and are poorly bound to serum proteins, as expected the concurrent administration of Cefepime and amikacin would not affect the pharmacokinetics of each other. Cefepime and Amikacin are not compatible with each other because of the solubility problem. To prepare the compatible fixed dose combination of Cefepime and Amikacin .To carry out the quality control of the formulation.

INTRODUCTION:

Cefepime and Amikacin for injection is a combination of Cefepime Hydrochloride and Amikacin Sulphate available as dry powder for reconstitution before use[1]. Cefepime and Amikacin for Injection, is supplied for intramuscular or intravenous administration in strengths equivalent to 625 mg, 1.25g and 2.5 g of cefepime and amikacin in 4:1 ratio[2].

Cefepime is a novel methoxyimino-aminothiazolyl cephalosporin with a quaternized N-methyl-pyrrolidine moiety at the 3' position conferring zwitterionic properties. Because of this the molecule penetrates the outer cell membrane of Gram-negative bacteria rapidly. In addition it is resistant to degradation by several plasmid and chromosomally-mediated beta-lactamases, for which it also shows very low affinity and no inducing capacity. It has good affinity for PBPs 2 and 3 of Escherichia coli and for PBP 3 of Pseudomonas aeruginosa. Its broad-spectrum of activity includes Gram-positive and Gram-negative pathogens. It is more active than cefotaxime or ceftazidime, against Enterobacteriaceae. The MIC90 for P. aeruginosa is higher than that of ceftazidime, but lower than those of cefpirome, cefoperazone and latamoxef. Other Gram-negative organisms, Haemophilus influenzae, Neiserria meningitidis, Neiserria gonorrhoeae, Moraxella catarrhalis are highly susceptible to Cefepime. Among Gram-positive species methicillin-susceptible Staphylococcus aureus and coagulase-negative staphylococci, whether beta-lactamase producers or not, Streptococcus pneumoniae and Streptococcus pyogenes are susceptible. Cefepime is active against cefotaxime- and/or ceftazidime-resistant Enterobacteriaceae. Only strains of P. aeruginosa producing large amounts of beta-lactamase may be resistant to both ceftazidime and Cefepime. In experimental infections such as meningitis, induced with various bacterial species in neonatal rats and chronic staphylococcal osteomyelitis in rabbits, Cefepime has shown good efficacy.To reduce the development of drug-resistant bacteria and maintain the effectiveness of Cefepime® and other antibacterial drugs, Cefepime should be used only to treat or prevent infections that are proven or strongly suspected to be caused by bacteria[3].Amikacin (a-mee-noe-GLYE-koe-sides) are used to treat serious bacterial infections. They work by killing bacteria or preventing their growth[4]. Documented studies report that the pharmacokinetic properties of cefepime before, during, and after amikacin co-administration and those of amikacin before, during, and after cefepime co-administration were in excellent agreement with the previously reported pharmacokinetic properties of these drugs[5-9].Amikacin are given by injection to treat serious bacterial infections in many different

parts of the body[10]. In addition, some Amikacin may be given by irrigation (applying a solution of the medicine to the skin or mucous membranes or washing out a body cavity) or by inhalation into the lungs[11]. Streptomycin may also be given for tuberculosis (TB). These medicines may be given with 1 or more other medicines for bacterial infections, or they may be given alone. Amikacin may also be used for other conditions as determined by your doctor. However, Amikacin will not work for colds, flu, or other virus infections. Amikacin given by injection are usually used for serious bacterial infections for which other medicines may not work. However, Amikacin may also cause some serious side effects, including damage to your hearing, sense of balance, and kidneys. These side effects may be more likely to occur in elderly patients and newborn infants. Cefepime and Amikacin is a sterile, dry mixture of cefepime hydrochloride, amikacin and a chemical vector . The chemical vector at an approximate concentration of 750 mg/g of the combination, is added to make them chemically compatible and control the pH of the constituted solution at 3.5 to 6.5. Cefepime hydrochloride is a semi-synthetic, broad spectrum, cephalosporin antibiotic for parenteral administration[12]. The chemical name is 1-[[(6R,7R)-7-[2-(2-amino-4-thiazolyl) -glyoxylamido] -2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0] oct-2-en-3-yl]methyl]-1-methylpyrrolidinium chloride, 7^{2-} (Z)-(O-methyloxime), monohydrochloride, monohydrate. Cefepime hydrochloride is a white to pale yellow powder with a molecular formula of $C_{19}H_{25}ClN_6O_5S_2$•HCl•H_20 and a molecular weight of 571.5. It is highly soluble in water. Amikacin sulfate is a semi-synthetic aminoglycoside antibiotic derived from kanamycin. D-Streptamine, O-3-amino-3-deoxy-a-b-glucopyranosyl)1>6)-O-[6-amino-6-deoxy-a-D-glucopyranosyl(1>4)]-N1-(4-amino-2-hydroxy-1-oxobutyl)-2 deoxy-(S)-,sulfate (1:2)(salt). It has the following molecular formula $C_{22}H_{43}N_5O_{13}$ • $2H_2SO_4$ with a molecular weight of 781.75. The combination dosage form is supplied as a sterile, colorless to light straw colored solution for IM or IV use. Cefepime and Amikacin is rapidly absorbed after intramuscular administration. Intramuscular Administration: In normal adult volunteers, average peak serum concentrations of cefepime is 49.9µg /ml and 5.5µg /ml after 1hour and 10 hours respectively and Amikacin about 21 µg/mL and 2.1 µg/ml are obtained 1 hour and 10 hours after intramuscular administration of 2500 mg , twice daily doses, respectively[13].

Intravenous Administration: Single doses of 2500 mg administered to normal adults as an infusion of cefepime and Amikacin produced levels of cefepime 85.8 µg/mL and 2.3 µg/mL and amikacin 18.0µg /ml and 0.75µg /mL at 1 hour and 10 hours post-infusion, respectively.

Eighty-four percent of the administered dose was excreted in the urine in 9 hours and about 94% with in 24 hours. Elimination of Cefepime and Amikacin is principally via renal excretion with an average (± SD) half-life of 2.0 (± 0.3) hours and total body clearance of 94-120.0 (± 10.0) mL/min in healthy volunteers. Cefepime and Amikacin pharmacokinetics are linear over the range 625 mg to 2.5 g. There is no evidence of accumulation in healthy volunteers receiving clinically relevant doses for a period of 5 days. The percentage time above the MIC (%T > MIC) of piperacillin for each regimen was calculated using Equation 1 from the individual PK parameters of each subject relative to a variety of pathogens including *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Bacteroides fragilis*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Staphylococcus aureus* and other common clinical pathogens. where T_{inf} = infusion time (1 h), $C_{1\ h}$ = concentration at the end of infusion (= C_{max}), $C_{0\ h}$ = concentration at the beginning of infusion, K = rate constant and = dosing interval. The modal MIC of piperacillin for each pathogen was determined by searching literature through Medline and selecting studies that reported MICs of P/T and were performed with isolates from the USA.[15–22] In addition, we compared %T > MIC for each regimen with that of each conventional dosing regimen (3.375 g q6h, 4.5 g q8h and 4.5 g q6h),[23,24] since %T > MIC is the best pharmacodynamic predictor of efficacy for ß-lactams. The %T > MIC values of conventional dosing regimens were calculated from the mean of the PK parameters in two studies published previously, using Equation 1.

A high performance liquid chromatographic procedure has been developed for the assay of a Cefepime and metronidazole mixture in aqueous solution[14]. The separation and quantitation were achieved on a phenyl column at ambient temperature using a mobile phase of 94.5:5.5 v/v water-acetonitrile containing 0.015 M pentane sulfonic acid sodium salt (adjusted to pH 3.4 with glacial acetic acid and then 4.0 with 45% potassium hydroxide) at a flow rate of 1.5 mL/min with detection of both analytes at 280 nm. The separation was achieved within 10 min with sensitivity in the ng/mL range for each analyte[15]. The method showed linearity for Cefepime and metronidazole in the 18.77 – 300.2 and 9.39 – 150.1 µg/mL ranges, respectively. Accuracy and precision were in the 0.52 – 2.40 and 0.63 – 2.77% ranges, respectively, for both analytes. The limits of detection for Cefepime and metronidazole were 125 and 63 ng/mL, respectively, based on a signal to noise ratio of 3 and a 10 µL injection[16].

MATERIAL AND METHODS:

Procedure :

All the ingredients are placed in the kenwood mixer, and operated for 15 min. If the mixture is not homogenous then again mix for 15 min. After that the accurately weighted quantity of mixer is transferred to vials which are previously sterized. Then the vials were sealed and labeled.

Quality Control of the Formulation:

Quality control testing of the formulation is carried out by using the standard testing procedures.

Description:

Transfer the dry powder in clear glass plate and observe the description of powder

and note down.

Identification: (By HPLC)

The chromatograms of test preparation obtained as directed in the assay exhibits two major peaks for Cefepime & Amikacin. The RT(retention time) of major peaks in the test preparation corresponds to that of standard preparation obtained as directed in assay.

Reconstituted Solution:

When powder is reconstituted with W.F.I., form clear and particles free solution.

Particulate Matter:

Inspect the vials for presence of particulate matter in powder. Visual inspection is done in presence of light against white and black back ground for presence of white particles, black particles, glass particles and fibers in powder.

pH

Check the pH of reconstituted solution by a suitable & calibrated pH meter and note the observation.

Estimation of Water Content:

Water content is estimated by the use of karl fisher method.

Assay

Mobile phase: Dissolve 5.76 g of Sodium 1-pentanesulphonate in water & dilute to 2000ml. Adjust pH to 3.4 with Glacial acetic acid, than with Potassium hydroxide TS to a pH 4.0. Prepare a filtered & degassed mixture of this solution & acetonitrile (94:6)

Chromatographic system:

Column– 3.9mm X 300mm, L1, 5micron

Wave length – 254nm

Flow rate – 2.0ml/min

Injected Volume – 20micro Liter

RSD – Not more than 2.0%

Std. Preparation:

Dissolve an accurately weighed quantity of Cefepime Hydrochloride (100mg) & Amikacin sukphate (50mg) in filtered D.D. water & dilute to50ml with filtered double distilled water. Take 5ml of above solution dilute to 10ml with Mobile phase.

Test preparation: - Dissolve an accurately weighed quantity of Cefepime & Amikacin inj. (80mg) in filtered double distilled Water. Take 5ml of above dilution & dilute to 10ml with filtered double distilled water.

Procedure:-

Separately inject equal volume of the standard preparation & test preparation in to the chromatograph. Record the chromatograms & measure the response of the measure peaks. Calculate the quantity in g per vial of Cefepime & Amikacin by the formula.

Cefepime HCL. Eqv. to Cefepime (in g/vial) =AT X dilution of standard X PC X
Average fill weight =AS X dilution of sample X 100

Where,

PC is the Potency of Cefepime in %

AT is the peak area of Cefepime test

AS is the peak area of Cefepime std.

Amikacin Sulphate eqv. to Amikacin (in g/vial)

AT X dilution of standard X PA X Average fill weight

AS X dilution of sample X 100

Where,

PA is the Potency of Amikacin in %, on as such basis

AT is the peak area of Amikacin

AS is the peak area of Amikacin std.

Stability Testing:

Accelerated stability study is performed just to know that how much degradation of the formulation occurs at accelerated conditions of temperature and relative humidity. Accelerated stability study was carried out by placing the formulation samples in stability chamber at a temperature of 40°c and relative humidity of 75 %. Study was carried out for 3 months. Analysis of the samples was carried out 3 times, 1st sample i.e. initial sample drawn on the start of study 2nd sample drawn after month and 3rd sample drawn in the end of 3rd month. There should not be more than 5% degradation in the potency of formulation.

RESULTS AND DISCUSSION:

Quality control of the Formulation:

Result of the quality control are shown in the certificate of analysis.

CERTIFICATE OF ANALYSIS

Venus Remedies Limited

PRODUCT: CEFEPIME & AMIKACIN 1.25g

Batch No. : CA012C

Mfg. Date : Nov. 2007 Date of Sampling: 22/11/2007

Exp. Date : Oct. 2009 Batch Size :100 Vials

TABLE-1

S. No.	TEST	SPECIFICATION	RESULTS
1	DESCRIPTION	White to pale yellow powder filled in colorless glass vial & sealed with rubber plugs & f/o Al. seal.	COMPLIES
2.	IDENTIFICATION	Should be positive for Cefepime hydrochloride & Amikacin sulphate.	COMPLIES
3.	CONSTITUTED SOLUTION	When reconstituted with WFI should be form clear & particles free solution.	COMPLIES
4.	PARTICULATE MATTER	Should be free from particulate matter when examine visually.	COMPLIES
5.	PH(10% solution inWFI)	3.0 to 6.0	5.47
6.	WATER	NMT 8.0%	5.8 %
7.	ASSAY Each vial Contains Cefepime hydrochloride USP equivalent to Cefepime Amikacin Sulphate USP equivalent to Amikacin	Claim / Limits 1.00g / 0.900g to 1.100g 0.250g / 0.225g to 0.275g	1.0306g

STABILITY STUDIES

Product Name : Cefepime and Amikacin for injection1.25g

Condition :40°c / 75%rh

Batch No. : CA012C

Date of Mfg. : Nov. 2007

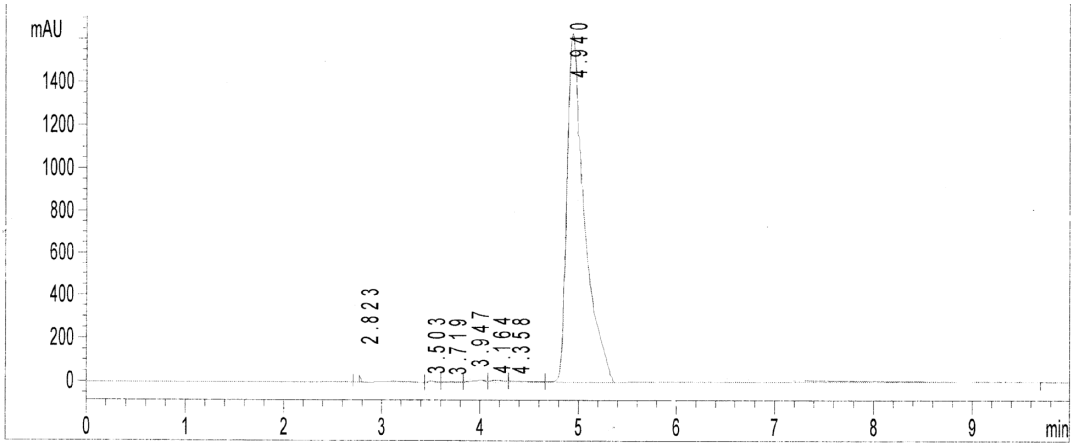
Date of Exp. : Oct. 2009

Date of initiating: 22/11/2007

Packaging : 20 ml glass vial

TABLE-2

Parameters	**Specifications**	**INITIAL**	**1Month**	**2Month**	**3Month**
1.Description	White or pale yellow crystalline powder.	A cream coloured powder.	A dark cream coloured powder.	A cream coloured powder with yellowish tinge.	A light yellow coloured powder.
2. Identification	Pass test	Passes test	Passes test	Passes test	Passes test
3. Sterility	Pass test	Passes test	Passes test	Passes test	Passes test
4. pH	3.0 – 6.0	4.56	4.51	4.46	4.38
5.Assay (by HPLC) CEFEPIME	90 -110 %	100.6%	100.1%	99.4%	98.8%
AMIKACIN	90 -110 %	100.4%	99.8%	99.1%	98.3%

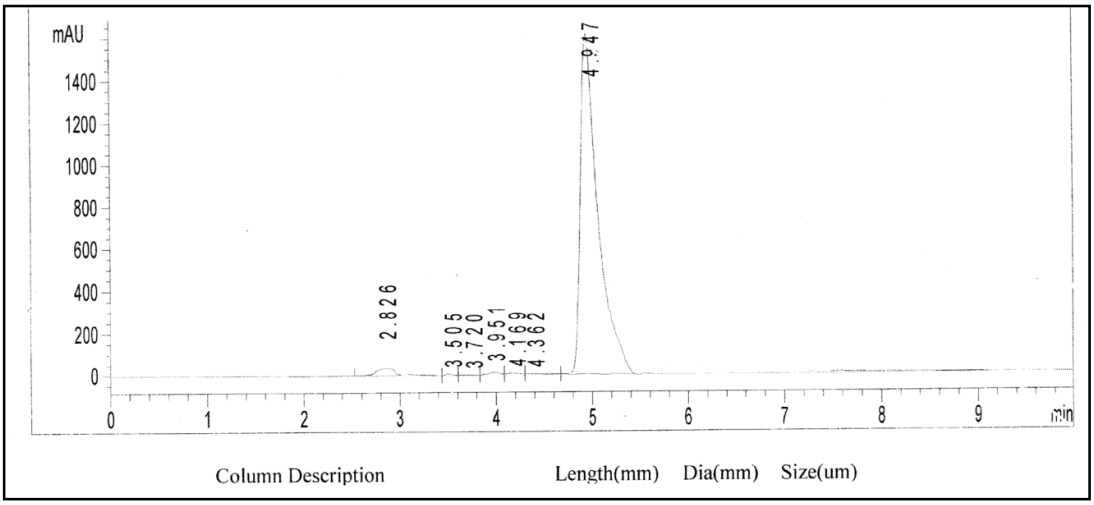


Column Description Length(mm) Dia(mm) Size(um)

Peak Results

RT(min)	Peak Area	Baseline	Area %
2.823	1352.638	VV	4.813
3.503	46.124	VV	0.164
3.719	48.019	VV	0.171
3.947	260.920	VV	0.928
4.164	113.712	VV	0.405
4.358	134.452	VV	0.478
4.940	26148.416	VB	93.041

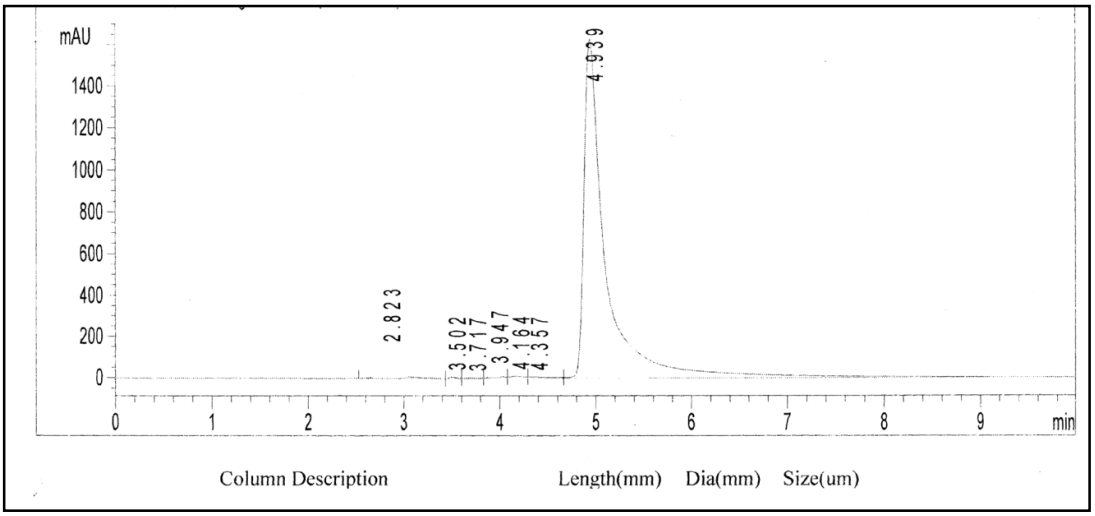
Fig. (a) Showing HPLC graph of initial sample.



Peak Results

RT(min)	Peak Area	Baseline	Area %
2.826	1349.753	VV	4.726
3.505	48.651	VV	0.170
3.720	48.807	VV	0.171
3.951	255.023	VV	0.893
4.169	118.699	VV	0.416
4.362	140.925	VV	0.493
4.947	26599.021	VBA	93.131

Fig. (b) Showing HPLC graph after 1 month



Peak Results

RT(min)	Peak Area	Baseline	Area %
2.823	1350.132	VV	4.729
3.502	46.999	VV	0.165
3.717	49.705	VV	0.174
3.947	260.251	VV	0.912
4.164	117.788	VV	0.413
4.357	141.670	VV	0.496
4.939	26585.258	VBA	93.112

CONCLUSION

The main emphasis of this project was to formulate the fixed dose combination of Cefepime and Amikacin and to carry out the quality control analysis of the formulation.

Formulation was developed by the addition of a suitable buffer (CA01). As its already discussed that the combination of Cefepime and Amikacin is not compatible with each other but the buffer made the combination compatible.

Analysis of the formulation was carried out by performing the HPLC of the formulation followed by estimation of water content by karl fisher's method. The result of the tests was within the limit. Accelerated stability study was performed by placing the sample in stability chamber. Result showed that the formulation is stable at accelerated conditions of temperature and relative humidity for three months period.

REFERENCES

1. Barriere SL, Ely E, Kapusnik JE, Gambertoglio JG. Analysis of a new method for assessing activity of combinations of antimicrobials: area under the bactericidal activity curve. J Antimicrob Chemother 1985;16:49–59.
2. Blaser J. Interactions of antimicrobial combinations in vitro: the relativity of synergism Scand J Infect Dis Suppl 1991;74:71–79.
3. Li RC, Schentag JJ, Nix DE. The fractional maximal effect method: a new way to characterize the effect of antibiotic combinations and other nonlinear.
4. Norden CW, Wentzel H, Keleti E. Comparison of techniques for measurement of in vitro antibiotic synergism J. Infect Dis 1979;140:629–633.
5. Moellering RC Jr, Eliopoulos GM, Allan JD. Beta-lactam/peptidoglycan antibiotic combinations: interactions and their mechanisms. Am J Med 1986;80(Suppl. 5C):30–34.
6. Butler JC, Dowell SF, Breiman RF. Epidemiology of emerging pneumococcal drug resistance: implications for treatment and prevention. Vaccine 1998;16:1693–1697.
7. Cappelletty DM, Rybak MJ. Comparison of methodologies for synergism testing of drug combinations against strains of Pseudomonas aeruginosa. Antimicrob Agents Chemother 1996;40:677–683.
8. Hamilton-Miller JMT. Rationalization of terminology and methodology in the study of antibiotic interaction. J Antimicrob Chemother 1985;15:655–658.
9. Jawetz E, Gunnison JB, Coleman VR. The combined action of penicillin with streptomycin or chloromycetin on enterococci in vitro. Science 1950;111:254.
10. Hook EW, Roberts RB, Sande MA. Antimicrobial therapy of experimental enterococcal endocarditis. Antimicrob Agents Chemother 1975;8:564–570.
11. Klugman KP, Friedland IR, Bradley JS. Bacterial activity against cephalosporin-resistant Streptococcus pneumoniae in cerebrospinal fluid of children with acute

bacterial meningitis Antimicrob. Agents Chemother 1995;39:1988–1992.

12. MacGowan AP, Wooton M, Hedges AJ, Bowker KE, Holt HA, Reeves DS. A new time-kill method of assessing the relative efficacy of antimicrobial agents alone and in combination developed using a representative β-lactam, aminoglycoside and fluoroquinilone. J Antimicrob Chemother 1996;38:193–203.
13. Novak R, Henriques B, Charpentier E, Normark S, Tuomanen E. Emergence of vancomycin tolerance in Streptococcus pneumoniae. Nature 1999;399:590–593.
14. Prichard MN, Prichard LE, Shipman CJ. Strategic design and three-dimensional analysis of antiviral drug combinations. Antimicrob Agents Chemother 1993;37:540–545.
15. Sanders CC, Sanders WE Jr, Moland ES. Decimal assay for additivity of drugs permits delineation of synergy and antagonism. Antimicrob Agents Chemother 1993;37:260–264.
16. Novak R, Henriques B, Charpentier E, Normark S, Tuomanen E. Emergence of vancomycin tolerance in Streptococcus pneumoniae. Nature 1999;399:590–593.