EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 11
Issue: 8
19-28
Year: 2024

COMPARISON OF FOUR METHODS IN COMPUTING AREA UNDER THE CONCENTRATION-TIME CURVE (AUC_{24}) OF VANCOMYCIN VERSUS INTEGRATION APPROACH (STANDARD) IN HEMODYNAMICALLY STABLE PATIENTS- A SINGLE-CENTERED RETROSPECTIVE STUDY

Yen Ping Ng[2]*, Yassini Maniam[1], Sam A. Thamby[3], Jing Ng[3], Boon Seng Tan[2], Jin Yi Choo[2], Cheng Hoon Yap[4]

[1]Therapeutic Drug Monitoring Department, Hospital Sultan Abdul Halim, Sungai Petani, Kedah, Malaysia.
[2]Faculty of Pharmacy, QUEST International University, Perak, Malaysia.
[3]Faculty of Pharmacy, AIMST University, Bedong, Kedah, Malaysia.
[4]Pharmacy Department, Hospital Raja Permaisuri Bainun, Ipoh, Perak, Malaysia.

***Corresponding Author: Yen Ping Ng**
Faculty of Pharmacy, QUEST International University, Perak, Malaysia.

Article Received on 01/06/2024 **Article Revised on 21/06/2024** **Article Accepted on 11/07/2024**

ABSTRACT

The updated guidelines recommend two approaches to estimate the AUC_{24} for patients receiving vancomycin; Bayesian-derived AUC_{24} monitoring or the first-order pharmacokinetic analytic equations. Bayesian approach is complex and unfamiliar to most pharmacists or clinicians. Several methods were developed to estimate AUC_{24} for vancomycin. However, which approach can produce a more accurate AUC_{24} remained unanswered. The main objectives of this study were to identify the method that able to provide a more accurate values of AUC_{24} and to explore the agreement of AUC_{24} estimated by the investigated methods to the integration model (standard). We conducted a retrospective analysis using the Electronic Hospital Information System from a tertiary hospital in Malaysia. Data pertaining to patients who received vancomycin treatment from 1st January 2021 to 31th of December 2022 were retrieved for analysis. The mean AUC_{24} of Simpson's Rule integrated method (standard) versus 2-Point trapezoidal, 3-Point trapezoidal, Pharmacokinetic Method 1(PK1) and Pharmacokinetic Method 2 (PK2) showed a statistically significant difference by 91.05 ± 40.88 mg·h/L, 116.95 ± 33.71 mg·h/L, 119.60 ± 40.91 mg·h/L and 38.73 ± 59.56 mg·h/L respectively, $p < 0.05$. None of the methods agree with the Simpson's Rule integrated method. The 2-Points, 3-Points, PK1 and PK2 methods also appeared to have some imprecision versus the Simpson's Rule Integration method, as 40%, 67.5%, 65 % and 12.5% of AUC_{24} estimates were outside the upper and lower agreement level of ±100 mg*h/L, respectively. The present study failed to demonstrate agreement of calculated AUC_{24} by the four methods to the Simpson integration approach (standard). All the approaches underestimated the AUC_{24}. Among the four methods, there is a modest variance of 38.73 mg*h/L in the PK2 values, which aligns most closely with the accuracy of Simpson's rule integration.

KEYWORDS: AUC_{24}, vancomycin, Simpson rule integrated approach, trapezoidal approach, log-linear approach.

INTRODUCTION

Vancomycin, an antibiotic indicated for Methicillin-Resistant Staphylococcus Aureus (MRSA) infections, is commonly used for serious infections.[1] Its efficacy is closely related to the ratio of 24-hour area under the serum concentration-time curve to the minimum inhibitory concentration (AUC24/MIC).[2] An AUC_{24}/MIC of 400 has been shown to predict better clinical and bacteriological outcomes. In order to determine the AUC_{24}, multiple blood samples are required. The 2009 consensus (Infectious Disease Society of America) recommends that to achieve the target for AUC-guided dosing using a first-order PK equation to estimate the AUC based on two concentrations sampled at 1–2 h after infusion and trough concentrations before the next dose.[3] The revised consensus guidelines for optimizing vancomycin doses suggest that maintaining the area under the concentration-time curve to minimal inhibitory concentration ratio (AUC/MIC) of 400–600 mg·h/L is the target pharmacokinetic/pharmacodynamic (PK/PD) index for efficacy. These guidelines recommend two approaches to estimate the AUC_{24} for patients receiving vancomycin; Bayesian-derived AUC_{24} monitoring or the first-order pharmacokinetic (PK) analytic equations.[4] Bayesian approach is complex and unfamiliar to most

pharmacists or clinicians, while the first order PK method requires the obtainment of two vancomycin concentrations at specific times, in order to calculate an AUC24, and this requires more dedicated time and resources.[4] Meanwhile, according to the second edition of Malaysia Clinical Pharmacokinetics Pharmacy Handbook (2019)[5], there are a total of four different methods which can be used to calculate the vancomycin AUC_{24}. All of these methods can be utilized in different situations giving approximately the same answer for the AUC_{24}.[5] However, which calculation approach is the most accessible that can produce a more accurate and reliable value for the AUC_{24} remained unanswered. Do the trapezoidal method (AUC two-points), AUC three-points or methods listed in the Malaysia Clinical Pharmacokinetics Pharmacy Handbook (2019)[5] able to produce a precise AUC_{24} compared to the standard (integration model)? The accuracy of AUC_{24} estimated by various methods as compared to the integral model (via Simpson' Rule) (Standard) remained unknown. It is acknowledged that rectangles and trapezoids approaches work better for linear functions while Simpson's Rule works better on curves.[6,7] As per our knowledge, this shall be the first research proposed to compare the accuracy and reliability of the AUC_{24} calculation models.

The main objectives of this study were to identify the method that able to provide a more accurate values of AUC_{24} by comparing the four methods (using 2 points method) versus integral approximation using Simpson's rule (as standard) and to explore the agreement of AUC_{24} estimated by the four methods to integral approximation (standard).

MATERIALS AND METHODS

This was a retrospective, observational study to compare the four methods for computing AUC_{24} of vancomycin in hemodynamically stable patients from 1st January 2021 to 31th of December 2022. The study was conducted in Sultan Abdul Halim General Hospital at Sungai Petani in the state of Kedah, Malaysia. Patients who age ≥ 18 years old; normal renal function; diagnosed with MRSA infection; minimum inhibitory concentration $(MIC)_{vancomycin} \leq 1$ mg/L; receiving intravenous vancomycin for ≥ 3 consecutive doses[8,9] from 1st January 2021 to 31st of December 2022; and haemodynamically stable were recruited into this study. Patients with unstable kidney functions; end stage renal disease; documented kidney transplant; anuric; pyelonephritis; allergy or hypersensitive to vancomycin; pregnant; and immunocompromised were excluded from this study. Unstable kidney function is defined as an acute decrease or increase in kidney function (GFR) over a period of hours, days, or even weeks. We follow the commonly used definition by which an increase or decrease in SCr of 0.5 mg/dL (44.2 µmol/L) or greater or a decrease or increase of 25% or greater in the GFR of patients with previous normal kidney function OR an increase or decrease of 1.0 mg/dl or greater in patients with chronic kidney disease (CKD) over a 24 to 48-hour period.[10]

The sample size required in this study was calculated according to Raosoft software (Raosoft, 2004).[11] The recorded total population of patients infected with MRSA and treated with vancomycin and documented to be hemodynamically stable in the specified time frame were 44 patients. The confidence level of the study was set at 95% with the margin of error of 5% and 50% respond distribution rate. The minimum sample size for the study was 40 patients.

Patient clinical data, including demographics, characteristics of vancomycin dosing regimens, and serum vancomycin through concentrations were recorded. Vancomycin administration times were identified from the therapeutic drug monitoring (TDM) requesting form in the Electronic Hospital Information System (eHIS). All vancomycin concentrations were analyzed by Particle-enhanced Turbidimetric Inhibition Immunoassay (PETINIA; Abbott Alinity Laboratories). Vancomycin concentrations of each patient were then use to calculate the AUC_{24} values.

The value of AUC_{24} of the two-point and three-point method is calculated utilizing the modified trapezoidal rule. In the two-point trapezoidal estimation method, for the patients who had received vancomycin dosing interval of 12 h the concentration of vancomycin was calculated at 0 and 12 h. The patient who had received vancomycin dosing interval of 8 h the concentration of vancomycin was calculated at 0 and 8 h. Whereas for the patient who had received vancomycin dosing interval of 6 h, the concentration was calculated at 0 and 6 h. In the three-point trapezoidal estimation method, for the patients who had received vancomycin dosing interval of 12 h the concentration of vancomycin was calculated at 0, 6 and 12 h. The patient who had received vancomycin dosing interval of 8 h the concentration of vancomycin was calculated at 0, 4 and 8 h. Whereas for the patient who had received vancomycin dosing interval of 6 h, the concentration was calculated at 0, 3 and 6. AUC two-points = $AUC_{0\text{-}12}$, $AUC_{12\text{-}24}$; AUC three-points = $AUC_{0\text{-}8}$, $AUC_{8\text{-}16}$, and $AUC_{16\text{-}24}$. AUC Two points (2P) is calculated using equation: $AUC_{24}: 2\left[0.5\ \frac{(Cmax+Cmin)}{2}\ (\Delta t)\right]$ x # of doses per day, while AUC Three points (3P) is calculated with the following equation: $AUC_{24}: 2x2\left[\left\{0.5\ \frac{(Cmax+Cmin)}{2}\ (\Delta t)\right\}+\left\{0.5\ \frac{(Cmax+Cmin)}{2}\ (\Delta t)\right\}\right]$ x # of doses per day. Pharmacokinetic 1 (PK1) method is calculated using Log method as the following: $AUC = \frac{Co-Cmin}{Ke}$, AUC_{24} =AUC ×dosing frequency. Pharmacokinetic 2 (PK2) method is calculated using the Linear-log method as the following: $AUC(inf) = t' \times \frac{Cmin+Cmax}{2}$; $AUC(elim) = \frac{(Cmax-Cmin)}{Ke}$; $AUC_{24} = (AUCinf + AUCelim) \times \frac{24}{T}$. Lastly, The formula for Simpson's Rule integrated method is as

following $AUC \int_0^{24} C\,dt = \left(\frac{R}{Vk}\right)\left[24 - \frac{1}{k}(1 - e^{-ke24}) + \frac{1}{k}(Co - Coe^{-ke24})\right]$; where $C\,dt$ = concentration of vancomycin in body at time t, R = dose, V = volume of distribution, k = elimination rate of renal and non-renal (kr + knr), ke = elimination rate constant, t = time. AUC_{24} values calculated utilizing the Simpson's Rule integrated method described above were compared with AUC_{24} estimates from two-point trapezoidal estimation method (2 Points), three-point trapezoidal estimation method (3 Points), method of PK1 and PK 2 as listed in the Malaysia Clinical Pharmacokinetics Pharmacy Handbook Second Edition 2019.[5]

Statistical analysis was performed using SPSS version 28.0 software. For outcome measures of continuous data, paired t-test was used to compare the mean scores of continuous variables. Wilcoxon's signed rank test was used for comparison of mean between experiment and control groups if data are not normally distributed. The Chi Square ($\chi 2$) test or Fisher's exact test was used to analyze dichotomous data. Bland-Altman analysis was used to determine the level of agreement of AUC_{24} estimated by the four methods to the integrated method. Descriptive statistics was used to summarize the baseline characteristics. Graphical data displays were used to summarize data. Data was analyzed for normality using skewedness and kurtosis. Differences were considered statistically significant if 2-tailed tests estimated at a *P* value of less than 0.05. Bias was estimated by calculating the median difference between the AUC24 estimates from the integral method (standard) and the four investigated methods. Precision was estimated by setting an upper and lower agreement limit of ±100 mg*h/L for AUC_{24} difference. These agreement limits were selected as an AUC_{24} of 500 mg*h/L is a reasonable therapeutic target and an absolute difference of more than100 mg*h/L would result in an AUC24 estimate outside the recommended AUC24 range of 400–600 mg*h/L (4). Bias and precision were graphically depicted using a non-parametric alternative to a Bland–Altman plot.[12]

The study was performed in accordance with the principles of the Declaration of Helsinki, as revised in Washington in 2013. This study attained approval from the Medical Research Ethics Committee (MREC), Ministry of Health Malaysia (NMRR ID-23-01264-YH8 (IIR). Institutional approval was obtained from the district health officer. All data collected were kept strictly confidential, and no identifiable information was collected.

RESULT AND DISCUSSION

A total of 53 patients with recorded vancomycin trough serum concentrations were identified during the study period, whom 9 patients had unstable renal function, 3 had concentrations not obtained at steady state and 1 had missing or incomplete data documented in the medical record. Thus, a total of 40 patients were recruited. Baseline characteristics of recruited patients and their pharmacokinetic parameters are shown in table 1.

Table 1: Baseline Characteristics of the Patients.

	Total (N=40)
Patient demographics	
Age in years *	54.7 ± 11.35
Age ≤50 years old	13 (32.5)
Age ≥50 years old	27 (67.5)
Weight in kilograms (kg) *	64.97 ±11.24
Female gender; n (%)	22 (55)
Race or ethnic background – no. (%)†	
Malay	27 (67.5)
Chinese	7 (12.5)
Indian	6 (15.0)
Wards	
Orthopaedic	18 (45)
Medical	14 (35)
ICU	6 (15)
Obstetrics and Gynecology	2 (5)
Serum creatinine in µmol/L *	86.13 ± 22.80
Creatinine clearance (ml/min)	73.73 ± 24.92
Documented MRSA infection; n (%)	100
Vancomycin dosing characteristics	
Dose in milligrams (mg) *	812.50 ± 185.62
Dose in mg/kg *	13.30 ± 3.01

Dosing frequency in hours *	10.25 ± 3.14
Number of doses per day *	2.53 ± 0.68
Dosing interval = q6h; n (%)	3 (7.5)
Dosing interval = q8h; n (%)	16 (40)
Dosing interval = q12h; n (%)	20 (50)
Dosing interval = q24h; n (%)	1 (2.5)
Pharmacokinetic parameters	
Ke in hours^{-1} *	0.0748 ± 0.0280
MIC in mg/L *	0.99 ± 0.61
Trough vancomycin (mg/L)	21.32 ± 11.98
Cmax in mg/L *	40.58 ± 12.59
Vd in litres *	43.38 ± 7.87

Abbreviations

*Plus-minus values are means ± SD ; ‡ Median (IQR), † Race or ethnic background was reported in the case note; q6h, every 6 h, etc.; Ke, elimination rate; Cmax, maximum concentration; Vd, volume of distribution; MIC, minimum inhibitory concentration.

The mean age of patients recruited in this study was 54.7 ± 11.35 years old. The youngest patients were 37 years old while the oldest patient being studied was 72 years old. This study observed more females than males, comprising of 22 females (55.0%) and 18 males (45.0%). The majority of the patients in this study were Malays with 27 (67.5%), followed by 7 Chinese (12.5%) and 6 Indians (15.0%). The mean total body weight of the patients was 64.97 ±11.24 kg and the mean serum creatinine is 86.13 ± 22.80 μmol/L. The mean creatinine clearance estimated by using Cockroft-Gault formula was 73.73 ± 24.92 ml/min.

The trough vancomycin concentrations, AUC_{24}-trapezoidal methods, pharmacokinetic methods, and Simpson's rule integration of each patient are shown in table 2.

Table 2: The trough vancomycin concentrations, AUC-trapezoidal method, Pharmacokinetics method, and Simpson's Rule Integration method of each patient.

Patient	Gender	Age	BW (Kg)	Dose (mg)	Dosing Interval (hour)	Trough (mcg/L)	MIC (mcg/ml)	SrCr (umol/L)	Crcl (ml/min)	Ke (h^{-1})	AUC 2 Point (mg·h/L)	AUC 3 Point (mg·h/L)	AUC PK1 (mg·h/L)	AUC PK2 (mg·h/L)	AUC Integration (standard) (mg·h/L)
1	M	69	70	750	8	19.56	0.75	95	64	0.0722	653.04	639.96	635.73	717.36	731.31
2	F	60	60	1000	12	13.16	0.25	128	39	0.0861	601.44	565.32	552.85	602.97	694.22
3	F	42	60	1000	12	7.84	2	52	118	0.1163	473.76	425.88	409.29	448.77	527.12
4	F	42	60	750	8	8.84	2	52	118	0.1381	426.36	397.5	387.76	441.06	461.37
5	F	42	60	750	6	63.06	2	52	118	0.0416	1727.4	1720.5	1714.42	2002.32	1858.93
6	F	42	60	750	8	10.84	2	52	118	0.1217	474.48	448.8	440.26	499.57	520.72
7	M	60	40	500	8	12.37	1.5	79	50	0.111	511.08	488.1	482.43	546.32	567.37
8	M	52	65	1000	12	14.27	0.38	98	72	0.0777	606.12	575.94	565.51	616.02	703.50
9	M	52	65	750	8	19.02	0.38	110	64	0.078	654.24	638.88	633.85	715.63	732.53
10	M	60	40	500	6	35.99	1.5	80	49	0.067	1077.96	1067.46	1065.67	1245.33	1178.53
11	M	59	70	1000	12	33.94	0.5	71	98	0.0392	1059.48	1049.94	1041.33	1129.62	1211.78
12	M	60	40	500	8	21.17	1.5	63	62	0.0769	722.4	705.6	696.75	787.05	806.18
13	M	37	67	1000	12	30.32	0.75	112	76	0.0444	983.52	966.48	960.36	1042.32	1132.01
14	F	42	55	500	12	9.72	2	78	72	0.0709	389.04	372.48	366.15	398.57	452.09
15	M	52	60	750	8	33.76	0.38	94	69	0.0531	1024.56	1013.16	1009.04	1137.11	1138.90
16	F	42	55	500	8	9.46	2	78	72	0.108	382.8	366.12	360.56	408.41	424.21
17	F	42	55	750	6	19.66	2	78	72	0.1145	705.12	685.08	679.13	796.65	768.37
18	F	72	50	750	8	34.99	1	62	57	0.0597	1096.92	1081.62	1076.88	1214.00	1224.15
19	F	72	52	750	12	19.02	1	101	37	0.0612	703.68	681.12	673.20	731.84	818.80
20	F	64	50	1000	8	41.81	0.25	97	41	0.0651	1346.28	1324.02	1316.59	1484.87	1505.07
21	F	64	50	500	12	28.97	0.25	92	41	0.0334	866.76	858.18	855.69	927.92	981.18
22	F	38	65	1000	12	6.74	1.5	64	108	0.1207	425.52	379.8	364.21	399.67	470.21
23	F	52	75	750	12	9.21	0.5	86	80	0.0781	392.52	372.78	365.94	398.65	455.33
24	F	52	75	750	8	19.02	0.5	86	80	0.07	627.96	616.14	612.43	690.92	702.90
25	M	41	75	1000	8	19.97	1.5	95	96	0.0837	707.88	688.98	682.80	771.28	792.09
26	M	41	75	1000	12	19	1.5	93	96	0.0578	684.6	665.1	659.17	716.22	796.52
27	M	60	80	500	12	29.96	1	93	84	0.041	947.52	754.92	928.78	1007.74	906.75
28	F	72	65	1000	12	15.83	0.38	113	41	0.0725	643.56	615.42	606.07	659.70	748.87
29	M	63	69	1000	8	17.53	0.75	81	81	0.0963	672.72	649.8	654.21	738.30	754.02
30	F	65	63	1000	24	17.59	1	125	39	0.0345	694.32	666.6	657.39	686.32	867.55
31	F	65	63	750	12	28.76	1	125	39	0.0387	894.36	882.66	879.07	953.60	1021.92
32	F	51	60	1000	12	29.4	1	118	47	0.0494	991.32	970.38	963.97	1046.58	1147.33
33	M	63	85	1000	12	7.68	1	89	90	0.0966	386.04	357.66	348.03	380.20	441.22
34	M	71	80	750	8	46.7	0.5	106	64	0.0315	1281.48	1276.5	1275.24	1435.42	1392.44
35	M	71	80	750	12	18.3	0.5	101	67	0.0456	599.88	589.14	587.28	637.27	692.66
36	F	58	50	1000	12	13	0.75	49	87	0.0969	654.84	606.3	589.68	644.25	747.99
37	F	58	50	750	8	17.2	0.75	54	79	0.1011	669.96	644.34	635.91	719.65	745.29
38	F	62	55	1000	12	13.1	0.5	60	75	0.0911	626.04	584.46	570.14	622.31	719.42
39	M	39	65	750	8	17.6	0.38	68	119	0.083	620.16	603.48	595.66	673.18	692.09
40	M	39	65	1000	12	18.44	0.38	115	70	0.0654	706.32	680.76	672.17	731.03	822.44
Mean ±SD		54.7 ±11.35	64.97 ±11.24	812.50 ± 185.62	10.25 ± 3.14	21.32 ± 11.98	0.99 ± 0.61	86.13 ± 22.80	73.73± 24.92	0.0748± 0.0280	742.836 ± 290.934	716.934± 294.673	714.29 ± 298.150	795.15± 342.995	833.885± 315.459

Comparison of AUC_{24} by Simpsons Rule Integration (Standard) method with the four investigated methods

Table 3: Comparison of AUC_{24} and Various Estimating Methods.

AUC_{24} by Integration method (mg/L*hr) (Standard)	AUC_{24} according to 2 Point method (mg/L*hr)		AUC_{24} according to 3 Point method (mg/L*hr)		AUC_{24} according to PK 1 method (mg/L*hr)		AUC_{24} according to PK 2 method (mg/L*hr)	
833.88 ± 315.46 Standard	742.84 ± 290.93	‡t = 14.09; $p < 0.001$	716.93± 294.67	‡t = 21.94 ; $p < 0.001$	714.29 ± 298.150	‡t = 18.47 ; $p < 0.001$	795.15 ± 342.99	‡t = 4.11; $p < 0.001$
Δ (mg/L*hr) Compared to Standard	91.05 ± 40.88	-	116.95± 33.71	-	119.60 ± 40.91	-	38.73 ± 59.56	-
% Δ Compared to Standard	11.11 ± 3.51	-	14.74 ± 3.38	-	15.28 ± 4.87	-	5.88 ± 6.88	-

The Comparison of the AUC_{24} calculated by Simpsons Rule Integration (Standard) method and the four estimating methods is shown in Table 3. The mean AUC_{24} of Simpson's Rule integrated method versus 2-point trapezoidal method shows a statistically significant difference by 91.05 ± 40.88 mg·h/L (P<0.001), which represents a difference of 11.11% (95% CI: -12.23 to -9.98). A statistically significant difference of mean was observed in AUC_{24} Simpson's Rule integrated method versus 3-point trapezoidal estimation method Simpson's Rule integrated method. The mean AUC_{24} of 3 point shows a statistically significant difference by 116.95 ± 33.71 mg·h/L (P<0.001) than the standard method, which represents a difference of 14.74 % (95% CI: -15,82 to -13.66). There is a significant difference of mean AUC_{24} in the Simpson's Rule integrated method versus PK1 calculated method (p<0.001). The mean AUC of PK1 demonstrated a statistically significant difference by 119.60 ± 40.91 mg·h/L than the standard method, which represents a difference of 15.28 % (95% CI: -16.83 to -13.72). A statistically significant difference of mean was observed in AUC_{24} Simpson's Rule integrated method versus PK2 calculated method. The mean AUC_{24} of PK2 shows a statistically significant difference by 38.73 ± 59.56 mg·h/L (P<0.001) than the standard method, which represents a difference of 5.88 % (95% CI: -8.08 to -3.60).

Agreement of estimated AUC_{24} using the four methods (investigated) compared to Simpson's Rule integrated method (standard) using Bland-Altman analysis.

Agreement Between Simpson's Rule integrated method (standard) and Various Estimating Methods are shown in Table 4. One sample T-test was used to analyze the mean differences of estimated AUC_{24} of the 2 point, 3 points, PK1, and PK2 equations to a value of zero (no difference). In general, (n = 40) all mean differences of AUC_{24} predicted by the 2-points, 3-points, PK1, and PK2 method equations were statistically significant with $p < 0.05$. None of the methods agree with the standard. Bland-Altman plot and linear regression were not performed since all differences observed were statistically significant, $p < 0.05$.

Table 4: Agreement Between Simpson's Rule integrated method (standard) and Various Estimating Methods.

One Sample T test	Mean difference	t	df	P- value	Lower	Upper
Standard	833.88	16.718	39	0.001	732.996	934.776
2 Points	742.84	16.148	39	0.001	649.791	835.881
3 Points	716.93	15.388	39	0.001	622.693	811.175
PK1	714.29	15.152	39	0.001	618.937	809.643
PK2	795.15	14.664	39	0.001	685.455	904.845

Pair-wise comparison	Mean difference	t	df	Correlation	P- value	Lower	Upper
Standard vs 2 Points	91.049	14.086	39	0.994	0.001	77.975	104.122
Standard vs 3 Points	116.951	21.944	39	0.996	0.001	106.170	127.731
Standard vs PK 1	119.595	18.487	39	0.993	0.001	106.510	132.680
Standard vs PK 2	38.735	4.113	39	0.987	0.001	19.685	57.784
2 Points vs 3 Points	25.902	5.628	39	0.995	0.001	16.592	35.212
2 Points vs PK 1	28.546	12.415	39	0.999	0.001	23.895	33.197
2 Points vs PK 2	-52.314	-5.681	39	0.997	0.001	-70.940	-33.689
3 Points vs PK 1	2.644	0.578	30	0.995	0.567	-6.608	11.896
3 Points vs PK 2	-78.216	-8.424	39	0.994	0.001	-96.996	-59.436
PK1 vs PK 2	-80.860	-10.87	39	0.998	0.001	-96.759	-64.961

Note: PK1; Pharmacokinetic Method 1; PK2: Pharmacokinetic Method 2 df; The degree of freedom for the single sample T-test is simply the number of valid observations minus 1, t; student t- statistic.

Assessing the bias and precision of estimated AUC_{24} using the four methods compared to Simpson's Rule integrated method (standard) using Bland-Altman analysis

Assessing the bias and precision of 2 points estimated methods compared to Simpson's Rule integrated method using Bland-Altman analysis.

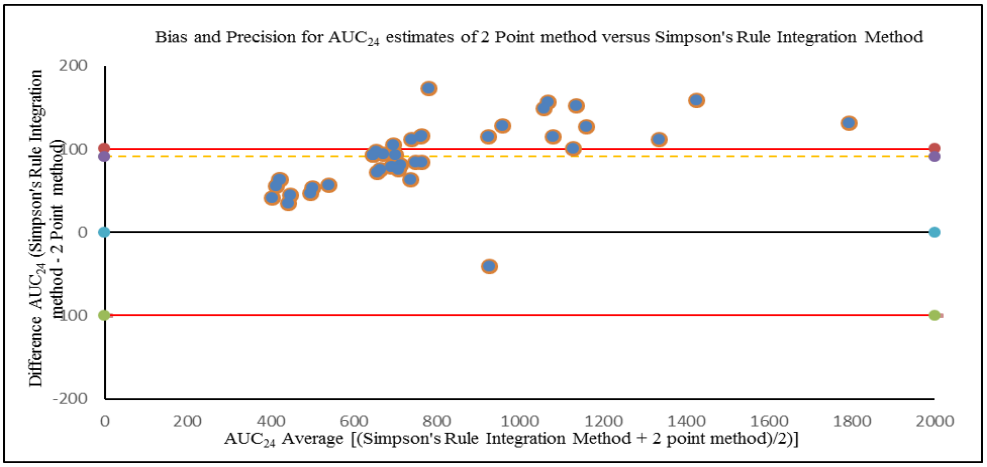

Figure 1: Graphical representation of bias and precision for AUC24 estimates for the 2-point method versus Simpson's Rule integrated method. Red dashed lines are the upper and lower agreement levels for AUC_{24} difference (± 100 mg*h/L) Orange dashed line is the mean AUC_{24} difference from Simpson's Rule integrated method: 91.05 ± 40.879 mg*h/L.

Figure 1 Shows the plots assessing the bias and precision of the AUC_{24} estimates from 2 points method. The 2 points appeared to have negative bias as the mean difference between the AUC_{24} estimates from the integration method and the 2 point was 91.05 mg*h/L, indicating the AUC_{24} estimates from 2-point method tended to be less than the integration method ($p < 0.001$).

Assessing the bias and precision of 3-points estimated methods compared to Simpson's Rule integrated method using Bland-Altman analysis.

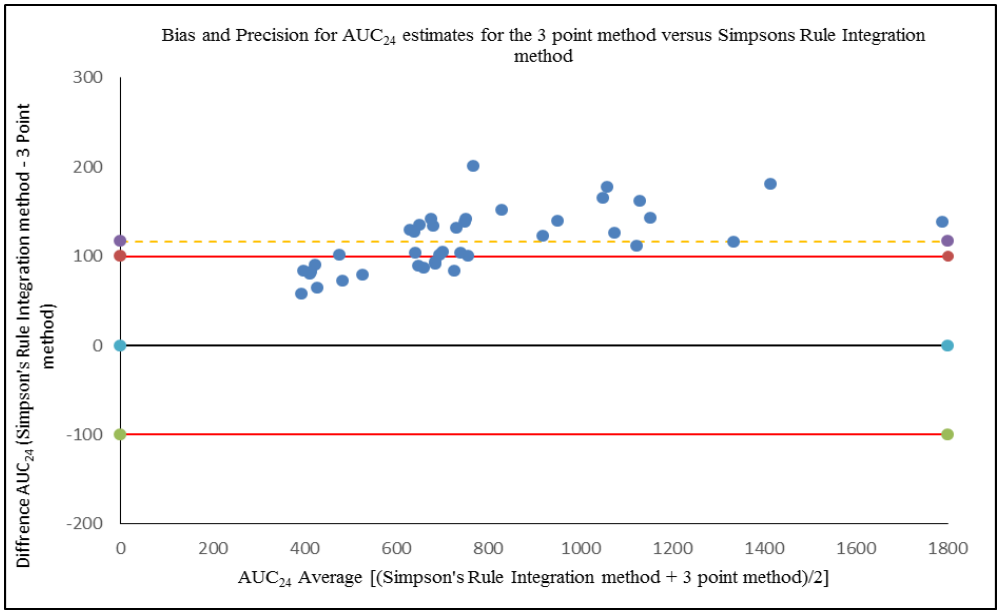

Figure 2: Graphical representation of bias and precision for AUC24 estimates for the 3 point method versus Simpson's Rule integrated method. Red dashed lines are the upper and lower agreement levels for AUC_{24} difference (± 100 mg*h/L) Orange dashed line is the mean AUC_{24} difference from Simpson's Rule integrated method: 116.951 ± 33.707 mg*h/L.

The 3-points method appeared to have a greater bias as the mean difference between the AUC_{24} estimates from the Simpson's Rule Integration method and the 3 Point method was 116.951 mg*h/L, indicating the AUC_{24} estimates from 3 point tended to be less than the Simpson's Rule Integration method ($p < 0.001$).

Assessing the bias and precision of PK1 estimated methods compared to Simpson's Rule integrated method using Bland-Altman analysis.

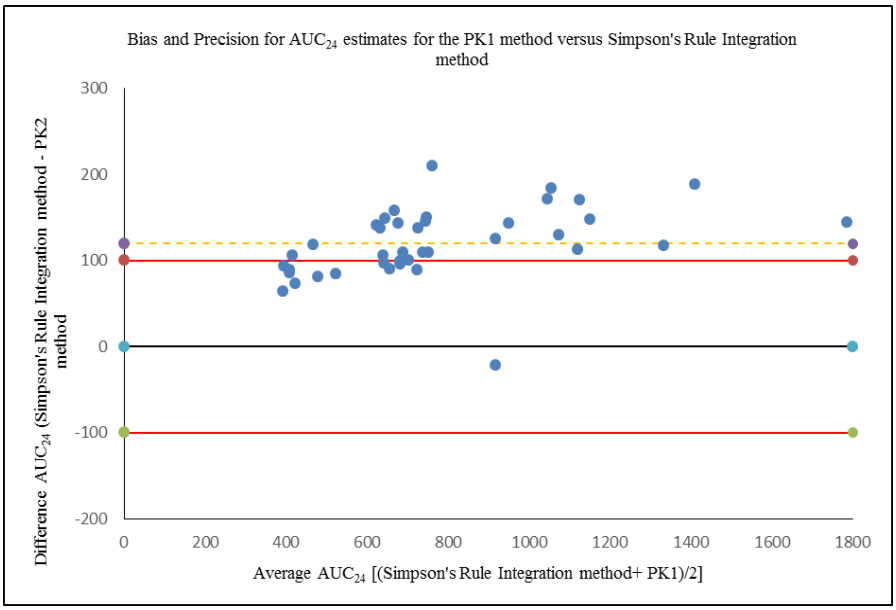

Figure 3: Graphical representation of bias and precision for AUC24 estimates for the 3-point method versus Simpson's Rule integrated method. Red dashed lines are the upper and lower agreement levels for AUC_{24} difference (± 100 mg*h/L) Orange dashed line is the mean AUC_{24} difference from Simpson's Rule integrated method: 119.595 ± 40.914 mg*h/L.

The PK1 method appeared to have a greater bias as the mean difference between the AUC_{24} estimates from the Simpson's Rule Integration method and the PK1 method was 119.595 mg*h/L, indicating the AUC_{24} estimates from PK1 point tended to be less than the Simpson's Rule Integration method ($p < 0.001$).

Assessing the bias and precision of PK2 estimated methods compared to Simpson's Rule integrated method using Bland-Altman analysis.

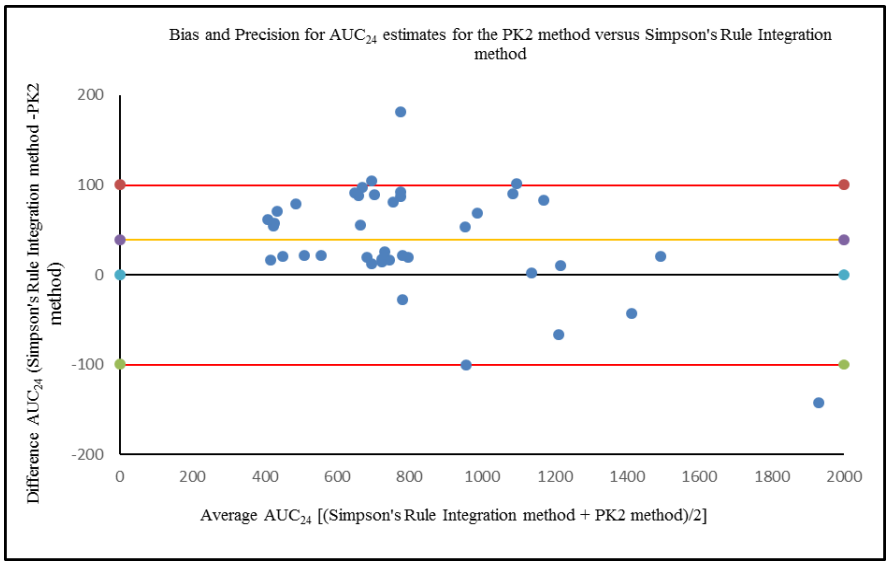

Figure 4: Graphical representation of bias and precision for AUC24 estimates for the 3-point method versus Simpson's Rule integrated method. Red dashed lines are the upper and lower agreement levels for AUC_{24} difference (± 100 mg*h/L) Orange dashed line is the mean AUC_{24} difference from Simpson's Rule integrated method: 38.735 ± 59.565 mg*h/L.

The PK 2 method appeared to have less bias as the mean difference between the AUC_{24} estimates from the Simpson's Rule Integration method and the PK2 method was 38.735 mg*h/L, indicating the AUC_{24} estimates from PK2 point tended to be less than the Simpson's Rule Integration method ($p < 0.001$).

The PK 2 method appeared to have less bias versus the 2-point, 3-point and PK 2 method as it had lower mean difference versus the Simpson's Rule Integration method (PK2, mean difference: 38.735 ± 59.565 mg*h/L vs 2 Point mean difference: 91.049 ± 40.879 mg*h/L vs 3 Point mean difference: 116.951 ± 33.707 mg*h/L and PK1 mean difference: 119.595± 40.914 mg*h/, $p < 0.001$).

Assessing the precision of the four AUC_{24} estimated methods compared to Simpson's Rule integrated method using Bland-Altman analysis.

The 2-point trapezoidal method, 3-point trapezoidal method, PK 1 and PK2 method also appeared to have some imprecision versus the Simpson's Rule Integration method, as 40% (16/40), 67.5% (27/40), 65 % (26/40) and 12.5% (5/40) of AUC_{24} estimates were outside the upper and lower agreement level of ±100 mg*h/L, respectively.

The PK 2 method appeared to have less imprecision, as fewer AUC_{24} estimates were outside the upper and lower agreement limits. [PK2: 12.5% (5/40) vs 2 point 40% (16/40), 3-point: 67.5% (27/40) and PK1: 65 % (26/40)].

DISCUSSION

Before calculus, the area was calculated by dividing the zones into very small bands and summing the respective areas (either Reimann sum or trapezoidal sum). The accuracy of the result is improved only by making the band smaller and smaller, bringing the result towards some limiting value.[13] In each of these cases, the area approximation got better as the width of the intervals decreased. This led to the concept of an integral as the limit of the area as the partition width tends toward zero. Simpson's Rule, named after Thomas Simpson though also used by Kepler a century before, was a way to approximate integrals without having to deal with lots of narrow rectangles. Its strength is that, although rectangles and trapezoids work better for linear functions, Simpson's Rule works quite well on curves.[14,15] According to a study conducted by Dhali et al. (2019)[16], it was noted that Simpson's 1/3 rule demonstrated the lowest error of 0.13% when applied to unevenly spaced data. In contrast, the 3/8 rule of Simpson yielded a higher error rate of 1.09%, and the Trapezoidal rule showed an error of 0.99% under similar conditions. In a separate study, a calculation difference of 6.23% was recorded for the Simpson rules. In general, the data show that the Simpson rule obtained a relatively low difference of 0.623 when compared to the Trapezium rule which yielded a value of 0.693 which means that the Simpson rule has a more accurate estimate value for AUC calculation using C- programming when compared to the Trapezium rules.[17] In a study conducted by Winnicka in 2019[18], an examination of three numerical integration methods on midpoint, trapezoidal, and Simpson's rules, revealed that the errors associated with Simpson's rule exhibited the fastest rate of decrease at 0.36%. The subsequent error reduction rates were observed to be 5.0% and 2.5% for the trapezoidal and midpoint methods, respectively. Thus, Simpson's rule remains as a reference.

To the best of our knowledge, this is the first study to compare the Simpsons Rule integration (Standard) method with AUC_{24} estimation methods, including the 2-points trapezoidal, 3-points trapezoidal, PK1, and PK2 methods. This study sought to assess various methods for calculating the AUC_{24}, specifically comparing the magnitude of difference in the standard Simpson's Rule integrated method with alternative AUC_{24} estimation methods. Our research demonstrates a notable disparity between Simpson's Rule and the PK1 log method, with a substantial difference of 119.60 ± 40.91 mg*h/L which represents a difference of 15.28 % (95% CI: -16.83 to -13.72) ($p < 0.001$), as opposed to the PK2 linear log method, which exhibits a comparatively lower difference of only 38.73 ± 59.56 mg*h/L which represents a difference of 5.88 % (95% CI: -8.08 to -3.60) ($p < 0.001$). 2-points trapezoidal method shows a difference of 11.11% (95% CI: -12.23 to -9.98) followed by 3-points trapezoidal method demonstrates a difference of 14.74 % (95% CI: -15,82 to -13.66). In an alternative investigation, three approaches were employed to compute the area under the concentration-time curve from 0 to the last measurable time point (AUC_{0-T}) in a G drug equivalence test: the Linear method, Linear/Log method, and Linear Up/Log Down method. The mean AUC values for the Linear method were 185.902 ±104.743 mg*h/L, while those for the Linear/Log method and Linear Up/Log Down method were 180.835 ±102.409 mg*h/L and 180.794 ± 102.479 mg*h/L, respectively. The Linear method demonstrated greater efficacy in producing stable results in small sample size tests or pre-tests, contrasting with the less frequently utilized Linear/Log method.[19] However, it is noteworthy that our study contraindicates the use of the PK2 method (Linear/Log method), despite its close approximation to the accuracy achieved by the Simpsons rule. This condition is influenced by factors such as the number of subjects examined, the timing of blood sampling, the extent to which the subjects represent the intended target population, and the inclusion of covariates that accurately reflect the characteristics of the overall population in the model.[20]

Among the four methods, there is a modest variance of 38.73 mg*h/L in the PK2 values, and it is worth noting which method aligns most closely with the accuracy of Simpson's rule integration, a variance that is not likely to significantly impact efficacy or toxicity outcomes. This is particularly noteworthy considering the suggested

AUC_{24} target for MRSA infection is in the range of 400–600 mg*h/L((assuming a vancomycin MIC_{BMD} of 1 mg/L) unless it is known to be greater or less than 1 mg/L by BMD [broth microdilution].[4] The PK2 linear log method demonstrates a reduced mean difference of 5.88% in comparison to the other AUC_{24} estimation methods of 2-points (11.11%), 3-points (14.74%), and PK1 (15.28%). Although the difference was statistically significant, clinically, the difference in AUC values (8.3%) was smaller than the 20% threshold for maximum acceptable imprecision.[21] Comparably, the FDA guidance (FDA 1997)[22] on an in-vitro–in-vivo correlation (IVIVC) states that the average absolute percent prediction error of ≤10% for Cmax and AUC0-∞ establishes the predictability of the IVIVC. In addition, the percent prediction error for an individual formulation should not exceed 15%. In this study, the predicted AUC_{24} value was well below the FDA limit except for the 3-Points trapezoidal estimation method.

The present study failed to demonstrate agreement of calculated AUC_{24} by the four methods to the Simpson integration approach (standard). All the approaches exhibit a negative bias (underestimating the AUC_{24}), as evidenced by the mean difference between AUC_{24} estimates obtained through the Simpsons rule integration method consistently being lower than the reference method of the Simpsons rule ($p < 0.001$). The PK2 Linear log method demonstrated comparatively less bias and lower imprecision when compared to the 2-point, 3-pont, and PK1 methods. In an investigation of 253 patients, the VancoPK single-concentration vancomycin calculator demonstrated reduced bias compared to the ClinCalc single-concentration vancomycin calculator in estimating the AUC. The VancoPK calculator showed a median (IQR) difference of 13 (44 to 23) mg*h/L, whereas the ClinCalc calculator had a median (IQR) difference of 53 (13–89) mg*h/L, with a statistically significant p-value of less than 0.001[23]. This estimate is consistent with the mean difference of 38.73 mg*h/L observed in our study and suggests there may be low bias with the PK2 Linear log method. Ondrsush (2022)[23] suggests approximately 89.7% of the AUC_{24} estimates from the VancoPK single-concentration vancomycin calculator would be within 100 mg*h/L of the 2PK reference. Our study found that 87.5% (35/40) of AUC_{24} estimates from the PK2 were within 100 mg*h/L suggesting similar imprecision and that it should be expected that 5–10% of AUC_{24} estimates from the PK2 linear log method may be more than 100 mg*h/L different from the Simpsons rule reference method.

CONCLUSION

In summary, the mean AUC_{24} of Simpson's Rule integrated method (standard) versus 2-Point trapezoidal (2P), 3-Point trapezoidal (3P), Pharmacokinetic Method 1 (PK1) and Pharmacokinetic Method 2 (PK2) showed a statistically significant difference by 91.05 ± 40.88 mg·h/L (11.11% ± 3.51%), 116.95 ± 33.71 mg·h/L (14.74% ± 3.3%), 119.60 ± 40.91 mg·h/L (15.28% ± 4.87%) and 38.73 ± 59.56 mg·h/L (5.88% ± 6.88%) respectively. All mean differences of AUC_{24} predicted by the 2-points, 3-points, PK1, and PK2 method equations were statistically significant with $p < 0.05$. None of the methods agree with the Simpson's Rule integrated method. Bland-Altman plot and linear regression were not performed since all differences observed were statistically significant, $p < 0.05$. The 2-Points, 3-Points, PK1 and PK2 methods also appeared to have some imprecision versus the Simpson's Rule Integration method, as 40% (16/40), 67.5% (27/40), 65 % (26/40) and 12.5% (5/40) of AUC_{24} estimates were outside the upper and lower agreement level of ±100 mg*h/L, respectively. AUC_{24} estimates from the PK2 method had less bias and better precision versus the 2-Points, 3-Points, and PK1 methods. All the methods may have clinical imprecision, as the 2-Points, 3-Points, PK1 and PK2 estimation methods had a clinical disagreement with Simpson's Rule Integration method in 12.5%, 25%, 30%, and 10% based on the AUC_{24} category.

In order to enhance the applicability of the study findings, it is recommended to conduct a future prospective study involving a larger cohort assessing clinical imprecision of the AUC_{24} estimate from the 2-Points, 3-Points, PK1 and PK2 methods. Future studies should evaluate vancomycin dosing in response to the growing occurrence of MRSA in the Malaysian population, focusing on effectively monitoring serum concentrations to enhance drug efficacy and reduce potential adverse events like nephrotoxicity.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ACKNOWLEDGEMENT

The authors would like to acknowledge the Director General of Health, Ministry of Health Malaysia for allowing to conduct this study and to publish the research finding.

REFERENCES

1. Hassoun A, Linden PK, Friedman B. Incidence, prevalence, and management of MRSA bacteremia across patient populations-a review of recent developments in MRSA management and treatment. Crit Care., 2017 Aug 14; 21(1): 211. doi: 10.1186/s13054-017-1801-3. PMID: 28807042; PMCID: PMC5557425.
2. Holmes NE, Turnidge JD, Munckhof WJ, Robinson JO, Korman TM, O'Sullivan MV, Anderson TL, Roberts SA, Warren SJ, Gao W, Howden BP, Johnson PD. Vancomycin AUC/MIC ratio and 30-day mortality in patients with Staphylococcus aureus bacteremia. Antimicrob Agents Chemother, 2013 Apr; 57(4): 1654-63. doi: 10.1128/AAC.01485-12.

Epub 2013 Jan 18. PMID: 23335735; PMCID: PMC3623342.
3. Rybak MJ, Lomaestro BM, Rotschafer JC, Moellering RC, Craig WA, Billeter M, Dalovisio JR, Levine DP. Vancomycin therapeutic guidelines: a summary of consensus recommendations from the infectious diseases Society of America, the American Society of Health-System Pharmacists, and the Society of Infectious Diseases Pharmacists. Clin Infect Dis., 2009 Aug 1; 49(3): 325-7. doi: 10.1086/600877. Erratum in: Clin Infect Dis., 2009 Nov 1; 49(9): 1465. PMID: 19569969.
4. Rybak MJ, Le J, Lodise TP, et al. Therapeutic monitoring of vancomycin for serious methicillin-resistant staphylococcus aureus infections: a revised consensus guideline and review by the american society of health-system pharmacists, the infectious diseases society of america, the pediatric infectious diseases society, and the society of infectious diseases pharmacists. *Am J Health Syst Pharm*., 2020; 77(11): 835-864.
5. Clinical Pharmacokinetics Pharmacy Handbook (2nd Edition). Pharmacy Practice & Development Division Ministry of Health Malaysia, 2019.
6. Atkinson, Kendall E. An Introduction to Numerical Analysis (2nd ed.), 1989. John Wiley & Sons. ISBN 0-471-50023-2.
7. Burden, Richard L.; Faires, J. Douglas. Numerical Analysis (7th ed.), 2000. Brooks/Cole. ISBN 0-534-38216-9.
8. Pai M, Neely M, Rodvold K, et al. Innovative approaches to optimizing the delivery of vancomycin in individual patients. Adv Drug Deliv Rev., 2014; 20(77): 50-57.
9. Belz SN, Seabury RW, Steele JM, et al. Accuracy of 4 free online dosing calculators in predicting the vancomycin area under the concentration-time curve calculating using a 2-point pharmacokinetic approach. Ann Pharmacother, 2022. 10600280221117256. doi:10.1177/10600280221117256
10. Mehta RL, Pascual MT, Soroko S, Savage BR, Himmelfarb J, Ikizler TA, Paganini EP, Chertow GM; Program to Improve Care in Acute Renal Disease. Spectrum of acute renal failure in the intensive care unit: the PICARD experience. Kidney Int., 2004 Oct; 66(4): 1613-21. doi: 10.1111/j.1523-1755.2004.00927.x. PMID: 15458458.
11. Raosoft Raosoft Sample Size Calculator. Raosoft, Inc., Seattle, 2004. http://www.raosoft.com/samplesize.html
12. Bland JM, Altman DG. Measuring agreement in method comparison studies. Stat. Methods Med. Res., 1999; 8: 135-160.
13. Nykamp DQ, "Calculating the area under a curve using Riemann sums." From *Math Insight*. http://mathinsight.org/calculating_area_under_curve_riemann_sums.
14. Cartwright, Kenneth V. (September 2017). "Simpson's Rule Cumulative Integration with MS Excel and Irregularly-spaced Data" (PDF). *Journal of Mathematical Sciences and Mathematics Education*, 2017; **12**(2): 1–9.
15. Kalambet, Yuri; Kozmin, Yuri; Samokhin, Andrey. "Comparison of integration rules in the case of very narrow chromatographic peaks". *Chemometrics and Intelligent Laboratory Systems*, 2018; **179**: 22–30. doi:10.1016/j.chemolab.2018.06.001. ISSN 0169-7439
16. Dhali, Md & Bulbul, Farhad & Sadiya, Umme. Comparison on Trapezoidal and Simpson's Rule for Unequal Data Space. International Journal of Mathematical Sciences and Computing, 2019; 5: 33-43. 10.5815/ijmsc.2019.04.03.
17. Tuah, A. N., Ibrahim, A.B., Dzulkifly, S., Mohammad Yusof, F., Awang Nor, R., & Ariffin, R. Analysis of the Area Under a Curve (AUC) using C-programming: Trapezium and Simpson rules techniques. Journal of ICT in Education, 2022; 9(1): 143-153. https://doi.org/10.37134/jictie.vol9.1.12.2022
18. Winnicka, Alicja. "Comparison of numerical integration methods." *System (Linköping)*, 2019.
19. Chen Chao, Zheng Qingshan, Li Lujin, Li Xue, Xu Ling. Comparison of the calculation approaches of AUC in non-compartment model pharmacokinetics[J]. Chinese Journal of Clinical Pharmacology and Therapeutics, 2020; 25(12): 1381-1387.
20. Buelga DS, del Mar Fernandez de Gatta M, Herrera EV, et al. Population pharmacokinetic analysis of vancomycin in patients with hematological malignancies. Antimicrob Agents Chemother., 2005; 49: 4934–4941. Santos Buelga et al., 2005.
21. Haeckel, R., & Wosniok, W. A new concept to derive permissible limits for analytical imprecision and bias considering diagnostic requirements and technical state-of-the-art. Clinical Chemistry and Laboratory Medicine, 2011; 49(4). doi:10.1515/cclm.2011.116
22. FDA (1997) Extended release solid oral dosage forms: development, evaluation and application of in vitro/in vivo correlations, September. US Department of Health, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), 1997.
23. Ondrush, N. M., Ademovic, R., Seabury, R. W., Darko, W., Miller, C. D., & Mogle, B. T. Comparison of vancomycin area under the concentration-time curve (AUC) using two-point pharmacokinetics versus two open-access online single-concentration vancomycin calculators. *Journal of clinical pharmacy and therapeutics*, 2022; *47*(12): 2223–2229. https://doi.org/10.1111/jcpt.13795