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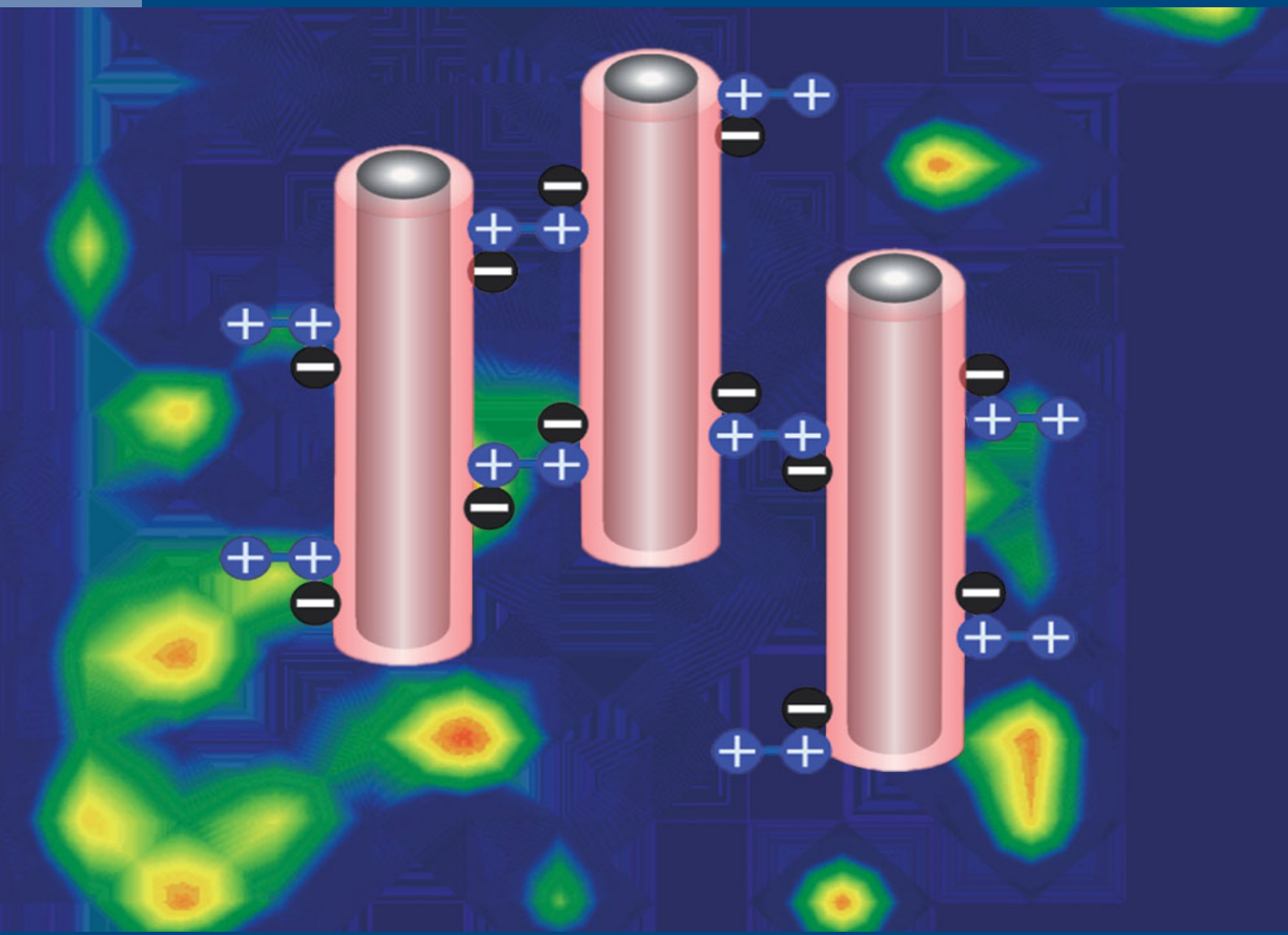

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Review Article

Modern methods for vancomycin determination in biological fluids by methods based on high-performance liquid chromatography – A review

Vancomycin is a glycopeptide antibiotic used in the therapy of severe bacterial infection. The monitoring of vancomycin levels is recommended because of its narrow therapeutic index and toxicity. This measurement is especially appropriate in patients with unstable renal functions, who receive high doses of vancomycin or present serious bacterial infections accompanied by important sequestration of liquids when it could be difficult to achieve the optimal therapeutic dose. Most of the methods for vancomycin determination in routine practice are immunoassays. However, chromatography-based techniques in combination with UV or mass spectrometry detection provide results with greater accuracy and precision also in complicated biological matrices. This review provides a detailed overview of modern approaches for the chromatographic separation of vancomycin in various biological samples and useful sample preparation procedures for vancomycin determination in various biological fluids.

Keywords: Biological fluids / High-performance liquid chromatography / Sample preparation / Vancomycin
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1 Introduction

Vancomycin (Fig. 1) is a tricyclic glycopeptide antibiotic introduced in 1956 [1, 2]. This antibiotic is effective against many gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis* [2–4], and *Clostridium difficile* [3, 5]. The antimicrobial effect of vancomycin is based on the inhibition of bacterial cell wall biosynthesis [6] upon binding to D-alanyl-D-alanine precursors [7].

Vancomycin therapy is especially indicated in the cases of severe staphylococcal and streptococcal infections that are resistant to penicillin and oxacillin or when allergies to penicillin are reported [3, 4, 8]. The earlier use of vancomycin was limited because of an association with toxicity and the availability of the probably less toxic semisynthetic penicillins. However, the use of vancomycin has recently increased owing to the growth of infections caused by MRSA and by other microorganisms that are resistant to penicillin [9].

Because of the narrow therapeutic index of vancomycin, the monitoring of vancomycin levels in patients is useful [2]. Under-dosing can lead to vancomycin resistance and ineffective therapy, while over-dosing may be associated with toxicity (nephrotoxicity, ototoxicity, and infusion-related toxicities) [2, 10]. For these reasons, vancomycin therapeutic level monitoring is recommended [10]. These serum levels should be determined in steady-state conditions, which could be reached approximately before the fourth dose (trough level) [10]. The minimum serum trough concentration should be higher than 10 mg/L (6.9 μmol/L) to avoid the development of bacterial resistance [10]. The optimal vancomycin serum trough concentration of 15–20 mg/L (10.4–13.8 μmol/L) is recommended if the minimum inhibitory concentration is 1 mg/L (0.69 μmol/L) [11]. Although not commonly provided, the peak concentration after 1–2 h after infusion should be in the range of 20–40 mg/L (13.8–27.6 μmol/L) [12]. The control of vancomycin levels is not performed routinely but is recommended in, for example, patients receiving aggressive dosing or in patients with high risks of nephrotoxicity and ototoxicity (treated by other nephrotoxins or ototoxic agents), unstable renal functions, and prolonged courses of therapy [10].

Vancomycin is poorly absorbed after oral administration; therefore, this means of administration is used for the local therapy of intestinal diseases. For the therapy of systemic infections, vancomycin is administered intravenously [3]. The

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Abbreviations: **ACN**, acetonitrile; **BAL**, bronchoalveolar lavage; **ECD**, electrochemical detector; **FA**, formic acid; **FLD**, fluorescence detector; **IS**, internal standard; **MeOH**, methanol; **MRSA**, methicillin-resistant *Staphylococcus aureus*; **PB**, phosphate buffer; **PDA (DAD)**, Photodiode array detector; **SPDE**, Solid-phase dispersive extraction; **TCA**, trichloroacetic acid; **TEA**, triethylamine buffer; **tR**, retention time

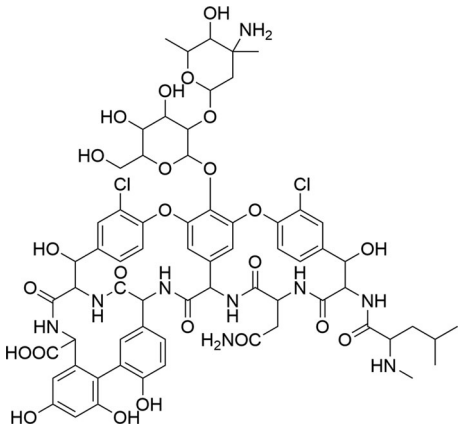

Figure 1. The structure of vancomycin.

serum half-life of this antibiotic in patients with normal renal function is approximately 6 h [1] and may be extended for up to 7 days in patients with renal insufficiencies. Approximately 10% of vancomycin is bound to proteins [1], and 80–90% of vancomycin is excreted by glomerular filtration in an unchanged form. Approximately 75% of the administered dose of vancomycin is excreted in urine within 24 h [5].

To the best of our knowledge, two reviews focused on vancomycin determination have been published in 2000 and 2007 [5, 39]. But in comparison with this work, the overviews and discussions of the LC and sample preparation methods for vancomycin measurement in biological samples were not so detailed and covered only the methods published until 2005. However, reviews published in 2000 and 2007 overlap with this review in a few of the earliest published methods because of some interesting information mentioned in these publications.

2 Sample pre-treatment

The determination of vancomycin is possible in different types of biological fluids. Most published methods are based on the analysis of vancomycin in human or animal serum [16, 18, 23, 29, 31, 32, 34, 37, 38, 40], plasma [8, 13–15, 17, 20, 21, 24, 28, 31, 33, 36, 37, 41], and urine samples [28, 32]. However, LC methods also enable the measurement of vancomycin in the cerebrospinal fluid [13] bronchoalveolar lavage (BAL) fluid [4], artificial perfusion fluid, lung tissue samples [14], tissues, bone samples [15–17] and vitreous and aqueous humor [18].

2.1 Protein precipitation

One of the methods of vancomycin sample preparation from serum and plasma is protein precipitation. This method is a relatively simple, cost-effective, and time-saving that uses organic solvents or acids for protein denaturation. Removing the proteins from the samples prevents interference with the assay and enables a longer lifetime of the chromatographic column. Table 1 gives an overview of the methods of sample preparation using protein precipitation.

Typical solvents for protein precipitation are acetonitrile (ACN) [4, 8, 11, 19, 20] and its mixture with water [13]. In addition, trichloroacetic acid (TCA) in concentrations of 5–30% [21–23] and its mixture with methanol [24] or methanol with 10% TFA [25] are extensively used as precipitation reagents.

Cheng et al. [21] studied the protein precipitation effect of TCA (5–35%) on the vancomycin recovery in rat plasma. Better sample recovery (about 70%) was achieved with 15–35% TCA than with ACN in various volume ratios with water (0.5:1, 1:1, 2:1, 4:1 v/v) where only less than 20% recovery was seen. [21]. A mixture of ACN with methanol was employed for protein precipitation in the method published by Muppidi et al. [26]. However, with this procedure, a low recovery ranging from 41 to 73% was reported for mouse plasma. Lower recovery values were achieved at higher concentrations of vancomycin, which may indicate insufficient extraction [26]. Zhang et al. [17] performed protein precipitation with methanol with a recovery value of approximately 100% for human plasma, bone, and fat tissue.

After protein precipitation and centrifugation, evaporation of the supernatant to dryness can be applied for sample pre-concentration, especially in HPLC–UV methods [4, 8, 11, 24, 26]. Dilution of the supernatant with water or a mobile phase before injection into the LC system is often reported. However, direct injection of the supernatant after the centrifugation step was also published [13, 14, 20–22].

Only several authors reported the filtration of the supernatant before injection into the LC system [25, 26]. Filtration is important mainly when a large series of samples is being measured. The supernatant obtained after protein precipitation is not so clean and can contain pieces of sediment or other small particles. The inclusion of a filtration step to the sample preparation procedure could be cost effective, especially for routine clinical laboratory practice, because of the extension of the column lifetime. However, it should be noted that testing for vancomycin retention into the filter is important. Syringe filters and microplates with filters made of different materials and with different pore-sizes are commercially available. In routine clinical practice, the use of filter microplates could be efficient because more samples are filtered simultaneously (96- or 384-well microplates) [27].

From Table 1, it is evident that only small sample volumes (25–300 μL) are required. It is also an important aspect of clinically suitable methods with respect to the patient. Larger volumes were only required in the method proposed by Abu-Shandi [8] and Tariq et al. [13]. However, providing a plasma sample of 1000 μL could be burdensome for the patient. Most of the listed methods achieved good recovery values. A relatively low mean recovery value of 65.6% (14.4% CV) was reported by Bijleveld et al. [22], 62.9% (5.6% CV) by Cao et al. [11] and 41–73% by Muppidi et al. [26]. A disadvantage of the protein precipitation method is that only the proteins are removed, while other endogenous compounds remain, which can still cause interferences or matrix effects in MS analyses. Therefore, greater emphasis on the development of separation methods is often required.

Table 1. Protein precipitation methods

Analyt	Internal standard	Matrix, sample volume	Precipitation reagent	Vancomycin recovery	Detection	Ref.
Vancomycin	Caffeine	Human plasma, 200 μL Mouse serum 200 μL Bronchoalveolar Lavage Fluid, 200 μL	ACN	85–90 %	UV	[4]
Vancomycin	Erythromycin	Human plasma, 500 μL	ACN	93 –102 %	FLD[e)]	[8]
Vancomycin	p-aminobenzoic acid	Human serum, 200 μL	ACN	62.9 % (CV 5.6%)	UV	[11]
Vancomycin Ceftriaxone	none	Rat plasma, 1000 μL Cerebrospinal fluid, 20 – 40 μL	ACN/H_2O (1:1 v/v)	101.48 % (CV 0.55%)	UV	[13]
Vancomycin	none	Artificial prefusion fluid, 300 μL Lung tissue, 300 μL	60% $HClO_4$	96–104 %	UV	[14]
Vancomycin	Aminopterin	Human plasma, 50 μL	MeOH	±100 %	MS/MS	[17]
Vancomycin Teicoplanin Daptomycin Colistin	Polymyxin B	Human plasma, 100 μL	ACN[a)]	70–110 %	MS/MS	[19]
Vancomycin	Vancomycin-des-leucin	Human plasma, 40 μL	ACN	106.3 % (CV 4.8 %)	MS/MS	[20]
Vancomycin Polymyxins	Dalbavancin	Human plasma, 50 μL	30% TCA[b)]	96–101 %	MS/MS	[21]
Vancomycin Amikacin Gentamicin	Kanamycin B	Human plasma, 25 μL	10 % TCA	65.6 % (CV 14.4 %)	MS/MS	[22]
Vancomycin	none	Human plasma, 100 μL Microdialisate	MeOH[c)] and 5 % TCA	86.7 % (CV 8.1 %) plasma 98.3 % (CV 15.4 %) microdialisate	UV	[24]
Vancomycin	none	Rat plasma, 100 μL	10 % TFA[d)] and MeOH (2:1 v/v)	unlisted	MS/MS	[25]
Vancomycin	Norvancomycin	Mouse plasma, 200 μL	ACN and MeOH	41–73 %	UV	[26]
Vancomycin	none	Human plasma, 200 μL	6 % $HClO_4$ and MeOH (85:15 v/v)	89.6–95.8 %	UV	[35]
Vancomycin	none	Human plasma, 200 μL	10 % $ZnSO_4$	unlisted	UV	[36]
Vancomycin	none	Human plasma, 100 μL	MeOH	103.1 % (CV 3.9 %)	ECD[f)]	[41]

a) ACN – acetonitrile.
b) TCA – tricholoroacetic acid.
c) MeOH – methanol.
d) TFA – trifluoroacetic acid.
e) FLD – fluorescence detector.
f) ECD – electrochemical detector.

2.2 SPE

SPE procedures are primarily connected with the HPLC–UV determination of vancomycin in biological samples. The use of SPE leads to cleaner sample extracts, resulting in greater selectivity and the decreased incidence of interferences. This method also enables the pre-concentration of the analyte. An overview of published SPE techniques for vancomycin extraction is provided in Table 2.

C_{18} SPE columns were chosen for vancomycin extraction, with average recoveries for serum and plasma of approximately 95% [15, 18]. Zhang et al. [16] used SPE in

Table 2. Solid phase extraction methods

Analyt	Internal standard	Matrix, sample volume	Cartridges	Conditioning	Elution	Recovery	Detection	Ref.
Vancomycin	Tinidazole	Human plasma, 500 μL Human tissues	Bond Elute C18	3 mL MeOH[a], 3 mL H_2O	2×300 μL ACN[b] and 0.05 mol/L KH_2PO_4, pH 4 (1:1 v/v)	76–112.5 %	UV	[15]
Vancomycin	Atenolol	Human serum, 200 μL	Strata X-C	1 ml MeOH, 1 mL H_2O, 1 mL 0.1% (v/v) FA[c]	1.5 mL 3% (v/v) NH_4OH in MeOH	78–94 %	MS^n	[16]
Vancomycin	Caffeine	Rat serum, 1000 μL Vitreous, aquaeous humour	Sep-Pak C18	3 mL MeOH, 3 mL H_2O	1 mL MeOH	97.5 % (CV 0.3%)	UV	[18]
Vancomycin Terbinafine Spironolactone Furosemide and their metabolites	none	Human plasma, 1000 μL Urine 1000 μL	Narc-2	3 mL MeOH, 3 mL H_2O (pH 7)	2 mL MeOH	85 % plasma 89 % urine	UV	[28]
Vancomycin	Cefuroxime	Hunam serum, 500 μL	Oasis® MCX	1 mL MeOH, 1 mL H_2O	1 mL MeOH with 5% NH_3	98.2–103.9 %	UV	[38]

a) MeOH – methanol.
b) ACN – acetonitrile.
c) FA – formic acid.

combination with LC–MS full scan analysis. Strata mixed mode SCX cartridges with a SCX phase were used. The recovery was 78–94% at three levels and no ion suppression caused by phospholipid ions was observed [16].

Moreover, sample preparation using SPE was performed for the simultaneous determination of vancomycin and other pharmaceuticals in a single method. This method was published by Baranowska et al. [28] for the simultaneous determination of vancomycin, terbinafine, spironolacton, furosemide, and their metabolites in human urine and plasma. Before the SPE of samples containing this mixture of analytes, the protein precipitation step with methanol and ACN (1:1 v/v) was necessary because of matrix interferences with the analytes. Narc-2 columns with a mixed mode sorbent were utilized for the extraction procedures owing to the high recoveries achieved for all the analytes (for vancomycin in plasma 85%, in urine 89%) [28].

Methanol is usually used as the elution solvent in combination with C_{18} cartridges [18, 28] or 3% v/v ammonium hydroxide in methanol is usually used with Strata SCX cartridges [16]. Mixtures of ACN and 50 mmol/L KH_2PO_4 (1:1 v/v) were also reported [15].

Compared to the protein precipitation method, the SPE technique presents several disadvantages. Larger volumes of sample (500–1000 μL) are required in most methods [15, 18, 28], although the method proposed by Zhang et al. [16] only required 200 μL. The SPE method is composed of several steps that have to be optimized (wide range of chemistries, solvents, pH, volumes, flow rates, etc.). The SPE cartridges sample volume capacity is also very important.

A trend in the SPE technique is miniaturization, which uses microplates with various sorbents. In combination with multichannel pipettes, this approach is faster, especially when large sequences of samples are processed [27]. To the best of our knowledge, this trend was not applied for vancomycin sample preparation. An advantage of SPE is the possibility of automation, which is helpful in clinical routine practices.

2.3 Solid-phase dispersive extraction (SPDE)

SPDE is a novel trend in sample clean-up for vancomycin determination in serum, which was investigated by Sakomoto et al. [29]. The principle of this method is based on the extraction of vancomycin from the serum sample by dispersing microparticles into a liquid sample, which enables better contact of the analyte with the sorbent, thus leading to higher extraction efficiencies. Special tubes with filters were required.

The microparticles were prepared from the commercially available Oasis® HLB solid-phase SPE cartridges by sorbent dispersion in water after conditioning with water and methanol. The microparticle suspension (100 μL, or at a concentration of 100 mg/mL) was directly added into the 500 μL serum sample (mixed with 500 μL of water and a single drop

Table 3. LLE after protein precipitation technique

Analyt	Internal standard	Matrix, Sample volume	Precipitation reagent	Solvents	Recovery	Detection	Ref.
Vancomycin	none	Human plasma, 1000 μL	$HClO_4$	Dichlormethane	unlisted	UV	[30]
Vancomycin	Ristocetin	Human serum, 50 μL Hunam plasma, 50 μL	15 % $HClO_4$	Hexane and tert-butyl methyl eter (1:1 v/v)	100.6–103.6 %	UV	[31]

of silicon antifoaming agent) and vortexed. The centrifugal unit filter was centrifuged at 2500 × *g* for 15 s, and the filtrate was eliminated. For the SPE gel washing, 1 mL of water was added to the filtrate two times. Finally, vancomycin was eluted by 60% methanol aqueous solution [29].

The conventional SPE method was applied after deproteinization (two times, and the obtained supernatants were combined) by 10% TCA which was used for the comparison. The recovery efficiency reached approximately 70%, or approximately 30% after dilution with water, a value similar to that obtained with the SPDE procedure [29]. The benefits of this method in comparison to SPE, as reported by Sakomoto et al. [29], are a higher recovery of approximately 90%, simplicity of use, speed of the procedure (only 15 min in comparison with 2 h for the conventional method), and seldom-observed matrix effects [29]. However, only low concentrations of vancomycin-spiked serum were tested (0.002–0.1 mg/L, 0.0014–0.069 μmol/L). Moreover, the authors did not mention the difficulty of the preparation of the sorbent suspension, which may be complicated for routine clinical practice and may render the method inaccurate. A significant practical advantage of SPDE conducted in clinical/hospital setting is that it is performed in a closed system, and there is therefore a lower risk of exposure to infection.

2.4 LLE after protein precipitation

To the best of our knowledge, the LLE technique without protein precipitation is not popular in vancomycin sample preparation. Del Nozal et al. [18] tested several solvents for the LLE of vancomycin (dichloromethane, chloroform, ethyl acetate, methanol, propanol, diethyl ether, or their combinations) as well as the addition of bases, acids, and salts, but the best recovery was not higher than 5% [18].

On the other hand, a suitable approach is the application of LLE after protein precipitation [30, 31]. For protein precipitation in both cases [30, 31], perchloric acid was added to the plasma or serum sample. After centrifugation, the supernatant was extracted with a non-polar organic solvent. Li et al. [31] used a mixture of hexane and *tert*-butyl methyl ether (1:1 v/v) and Lukša et al. [30] added dichloromethane for the extraction of vancomycin. The aqueous layer was injected into the column. The combination of these two techniques led to the high analytical recovery of vancomycin of 100.6–103.6%, as published by Li et al. [31], and surprisingly, a very low volume of sample (50 μL) was used. However, in the method proposed by Lukša et al. [30] a high sample volume (1000 μL) was required. More information provides Table 3.

A disadvantage of the LLE method for vancomycin extraction is the use of toxic solvents such as dichloromethane, hexane, and *tert*-butyl methyl ether; in addition, the automation of the LLE method is difficult.

2.5 On-line sample extraction

Several methods in which the sample pre-treatment step is not required, and direct sample injection into the chromatographic system is possible, have been described. Cass et al. [32] published a fully automated LC–MS/MS method with on-line serum and urine sample extraction, with one column used for extraction and two analytical columns for faster analysis. This method simultaneously allows analysis in one column and equilibration in the other. The sample preparation consisted only of the centrifugation of the serum or urine sample (90 μL) mixed with the internal standard (10 μL, 20 μg/mL in 10% formic acid) at 1500 × *g* for 10 min. The supernatant was directly injected into the LC system [32]. The short running time of the method (15 s of sample extraction, 90 s of chromatography) and the low sample volume of 90 μL are the notable advantages of this system. However, this system composition could be complicated for routine clinical practice, especially in small laboratories. In addition, only low concentrations of vancomycin in the samples were tested. Moreover, this method requires additional equipment for the HPLC system: two column-switching devices (six-port and ten-port) and three HPLC columns.

Another on-line method for the extraction of vancomycin from plasma was proposed by Saito et al. [33]. This method follows on the work published by Demotes-Mainard et al. [34]. The chromatographic system consisted of two columns in the ion-exchange mode and connected in tandem and one trap C_{18} column and one C_{18} phase separation column linked through a six-port valve. Only the dilution of the plasma sample (20 μL) by the mobile phase (67 mmol/L phosphate buffer, pH 5.3), centrifugation, and filtration using Milex-LH filters are required for sample preparation. The recovery of vancomycin from rat plasma reached 101%, and the separation was completed in 15 min [33]. This system composition requires four columns and a six-port switching valve.

Table 4. Ultrafiltration

Analyt	Internal standard	Matrix, sample volume	Ultra-filtration device	Recovery	Detection	Ref.
Vancomycin	Vancomycin-glycin	Human serum, 75 μL	Amicon Ultra-0.5 mL 10k (10 kDa nominal molecular cut-off)	99–104 %	MS/MS	[23]
Free vancomycin	none	Human plasma, 500 μL	Amicon Centrifree Microportition device (10 kDa nominal molecular cut-off)	97.7–104.1 %	UV	[35]
Free vancomycin	none	Hunam plasma, 500 μL	Hollow fiber (molecular cut-off 10 kDa, wall tickness 150 μm, inner diameter 1000 μm	96.7–100.7 %	UV	[36]
Free Vancomycin	none	Human plasma 300 μL, Human serum, 300 μL	Nanosep Omega PES (molecular cut off 10 kDa)	97.4 % (CV 1.9 %)	UV	[37]
Free Vancomycin	Cefuroxime	Hunam serum, 500 μL	Centrifree® (molecular cut off 30 kDa)	98.2–103.9 %	UV	[38]

The on-line sample extraction technique is a fast technique in general. It does not require time-consuming sample preparation procedures, but the injection of untreated biological samples could lead to the short lifetime of the columns. It could be costly for clinical practice when a large number of samples is analyzed. However, Cass et al. [32] reported that the lifetime of the extraction column was greater than 1000 samples, and the lifetime of the analytical column was found to be of approximately 600 injections. The presence of formic acid in the samples suppressed the growth of bacteria, and any solid particles were eliminated by centrifugation [32].

2.6 Ultrafiltration

Ultrafiltration as a vancomycin extraction technique is mainly used when the determination of free vancomycin is desired. This method allows separating free vancomycin from the protein-bound vancomycin fraction in biological samples. Therapeutic drug monitoring is based on the total drug concentration in human serum or plasma. However, it is known that only the non-protein-bound (free) portion of the total concentration of vancomycin has antimicrobial activity [35–37].

Ultrafiltration is a notably simple method in which the separation of free vancomycin is carried out using an ultrafiltration device (centrifugation tube). Most often, serum or plasma volume samples of 500 μL or less are subjected to ultrafiltration in a device containing a membrane with a nominal molecular weight limit of 10 or 30 kDa and centrifuged between 10 and 30 min at 1000–3000 × *g* at different temperatures (4–37°C) [35–38]. In this technique, setting the optimal centrifugation force, centrifugation time, temperature, pH, material type and nominal molecular weight limit of the ultrafiltration membrane is important because of the significant influence of these parameters on the free drug concentration [35, 37]. Table 4 gives an overview of the methods of sample preparation using ultrafiltration.

König et al. [23] published a method consisting of protein precipitation followed by ultrafiltration for total vancomycin extraction. In this method, only a small volume of serum sample (75 μL) is used, but the duration of entire process is too long (45 min) in comparison with other sample preparation methods. However, the authors did not discuss the possibility of handling larger sequences of samples in a single sample preparation procedure. This approach could significantly reduce the time required for the preparation of one sample. The reason for the inclusion of the ultrafiltration step, as reported by the authors, was to maximize the robustness of the method [23].

Another interesting technique connected with ultrafiltration is the application of a hollow fiber. Zhang et al. [36] observed the effect of plasma conditions (from different patient diseases) on the volume ratio of the ultrafiltrate to sample solution.

Their results showed that the different plasma conditions (total protein levels and osmotic pressure of the plasma) had a significant and simultaneous impact on this ratio and on the free vancomycin concentration [36]. As a solution to this problem, hollow fiber centrifugal ultrafiltration was tested. The hollow fiber (cut into 15 cm segments) with a molecular cut-off of 10 kDa was placed into a glass tube, and 500 μL of plasma sample were added. After incubation in a water bath (37°C) for 10 min and centrifugation at 1250 × *g* for 10 min at 37°C, the ultrafiltrate was pushed out from the hollow fiber lumen with a syringe. Zhang et al. [36] reported that the volume ratio of the ultrafiltrate to sample solution

was better controlled by the inner glass tube and hollow fiber and was less affected by the plasma conditions. The authors mentioned the potential suitability of this method for routine therapeutic drug monitoring practice. However, this potential could be discussed because of the difficulty of this method for manual handling.

3 Determination of vancomycin

For vancomycin determination in human liquids, two types of analytical methods are mainly used: immunoassays (including fluorescence polarization immunoassay, RIA, and enzyme-multiplied immunoassay) and LC techniques. In therapeutic drug monitoring practice, primarily immunochemical methods are applied [20, 39] owing to their simplicity, rapidity and the commercial availability of kits or automated analyzers. However, the immunochemical methods have several disadvantages, such as relatively high cost and the possibility of cross-reactivity with other vancomycin-related substances (vancomycin degradation products or compounds formed during the production process) [20, 39]. Most of the recently published methods for the determination of vancomycin in biological samples are chromatographic techniques. These techniques are more sensitive and specific and enable the detection of lower concentrations of analytes with higher accuracy and precision [11].

3.1 Chromatographic separation

The techniques for the chromatographic separation of vancomycin in biological fluids are often based on a reverse phase mode with a polar mobile phase. The most widespread stationary phase consists of octadecyl carbon chains (C_{18}) bonded to silica, with particle sizes in the range of 1.7–10 μm, which are suitable for UHPLC systems [4, 8, 11, 14, 17, 18, 24–26, 30, 32–38]. Except for full porous particles, core–shell technology consisting of a solid core surrounded by a porous silica outer layer was also applied as a new trend in column particle technology [19]. The use of C_{18} phases in routine practice for vancomycin chromatographic separation enables the shared use of this column in other routine methods.

Because of the high polarity and hydrophilicity of vancomycin molecules, a HILIC stationary phase was also tested. However, HILIC technology is suitable mainly for smaller molecules. Oyaert et al. [20] achieved a good retention time (2.7 min, total runtime 5 min) for vancomycin, with a LOQ value of 0.3 mg/L (0.21 μmol/L) and linear range of 0.3–100 mg/L (0.21–69 μmol/L). This method is therefore optimal for clinical practice.

Sakamoto et al. [29] used ZIC® HILIC, which is a type of HILIC with a zwitterionic stationary phase covalently attached to porous silica. In this article, the authors mentioned the decreasing peak area of vancomycin when 60% methanol was used in the vancomycin extraction method and the C_{18} phase was applied in the separation process. They solved this problem by using ZIC® HILIC, but the retention time of vancomycin was long (15 min) and the published linear range (0.002–0.100 mg/L, 0.0014–0.069 μmol/L) was not adequate for therapeutic drug monitoring. Moreover, they did not mention the testing of higher concentration levels.

Other chromatographic RP mode phases, such as C_8 [15, 16, 23, 40], C_1 [13] and ion-pair RP chromatography [22], were also applied. In the normal-phase mode, the amino propyl phase was exploited [31].

The isocratic and gradient separation methods were utilized for vancomycin determination. The mobile phase composition in the isocratic mode was based on the selected determination technique. For UV detection, the most often used mobile phase was a mixture of a phosphate buffer (in molarities ranging from 0.005–0.067 mol/L and pH ranging from 2.8–7) in various ACN ratios [4, 14, 18, 30, 31, 33, 36]. Del Nozal et al. [18] studied the effect of the pH of the phosphate buffer, the volume of ACN, and the column temperature on the retention time of vancomycin. The influence of the pH was studied in the range from 3 to 7.5 with a mobile phase composed of 0.05 mol/L ammonium dihydrogen phosphate buffer and 10% ACN. These researchers determined that the retention time was the same from pH 3 to pH 5, but for pH higher than 5, the retention time increased. To investigate the effect of ACN concentration in the range of 9–15%, 0.05 mol/L phosphate buffer (pH 4) was used. Increasing portions of ACN caused decreasing retention times [18]. Similar results were reported by Hagihara et al. [4].

For a connection with MS detection, the typical composition of the mobile phase was 0.1% formic acid or acetic acid in water and ACN (9:1v/v) [16, 25]. The mobile phase composition for gradient elution was similar to that used in the isocratic mode. Moreover, a mixture of ACN and formic acid was the most frequent choice of mobile phase for MS detection [17, 19, 20, 23, 28, 29, 32]. For UV determination, a combination of buffers and organic solvents was often used [11, 15, 24, 38].

Table 5 gives an overview of the chromatographic separation conditions.

3.2 Detection

3.2.1 UV detection

UV detection is commonly used for the quantification of vancomycin in biological fluids [4, 11, 13–15, 18, 24, 26, 28, 30, 31, 35, 36, 38, 40]. The wavelength of maximum absorbance for vancomycin is 198 nm [18]. However, in this region, interference can occur with other substances present in the serum [40] or with the solvents used for sample preparation or as the mobile phase (e.g., ACN has maximum absorbance 195 nm). This wavelength was used by Del Nozal et al. [18] for vancomycin determination because it improves the sensitivity of the method. To avoid interference peaks, a clean-up of the serum sample by SPE was necessary [18]. A wavelength of 198 nm was also used by Hagihara et al. [4] for

Table 5. An overview of chromatographic conditions and determination of vancomycin

Analyte	Internal standard	Matrix	Column[a)]	Mobile phase[b)]	Detector	tR[m)] (min)	LOQ (mg/L)	Lin. range	Ref.
Vancomycin	Caffeine	Hunam plasma	Spherisorb C18 ODS (150×4.6 mm, 5 μm)	0.05 mol/L $NH_4H_2PO_4$ and 11 % ACN isocratic elution, ambient temperature[b)]	UV 240 nm	8.5	1.0	1.0–80	[4]
Vancomycin	Caffeine	Bronchoalveolar Lavage Fluid	Spherisorb C18 ODS (150×4.6 mm, 5 μm)	0.05mol/L $NH_4H_2PO_4$ and 11 % ACN with 1mol/l NaOH (0.2%), pH 5.5, isocratic elution, ambient temperature	UV 198 nm	14.4	0.1	0.1–10	[4]
Vancomycin	Erythromycin	Human plasma	μBondapak C18 (300×4 mm, 10 μm)	A: 0.005 mol/L KH_2PO_4, pH 6.3 B: MeOH[h)] gradient elution, ambient temperature	FLD[g)] ex 225 nm, em 258 nm	16	0.005	0.005–1.0	[8]
Vancomycin	*p*-Aminobenzoic acid	Human serum	ACQUITY BEH C18 (50×2.1 mm, 1.7 μm)	0.005 mol/L KH_2PO_4, pH 2.5 and ACN gradient elution	PDA 230 nm	2.6	1.0	1.0–100	[11]
Vancomycin Ceftriaxone	none	Rat plasma Cerebrospinal fluid	Betacil C1 column (250×4.6 mm, 5 μm)	ACN and TEA[l)] buffer, pH 3.5 (20:80 v/v), isocratic elution	UV 280 nm	2.6 ± 1.1	0.56	62.5–375	[13]
Vancomycin	none	Artificial prefusion fluid Lung tissue	Nucleosil 120 C18 (150×4 mm, 5 μm)	0.05 mol/L $NH_4H_2PO_4$, pH 4 and ACN (92:8 v/v), isocratic elution, 40°C	UV 220 nm	8.5	0.1	0.1 – 2 2 – 15 15 – 250	[14]
Vancomycin	Tinidazole	Human plasma Human tissues	Hypersil BDS C8 (100×4.6 mm, 3 μm)	A: 0.005 mol/L KH2PO4 buffer, pH 2.8 B: ACN gradient elution 23 – 25°C	UV 282 nm	7.6	0.5	0.5–75	[15]
Vancomycin	Atenolol	Human serum	ACE-3-C8 (50×3.0 mm, 3μm)	0.1 % FA[f)] and ACN[c)] (9:1 v/v) isocratic elution, 40°C	LTQ-Orbitrap, ESI (+)[e)]	5	0.005	0.05–10	[16]
Vancomycin	Aminopterin	Human plasma Human bone Fat tissue	Luna C18(2) (50×2 mm, 5 μm)	A: 0.05 % formic acid in water B: methanol gradient elution	MS/MS, ESI (+)	2.39	0.05	0.05–50	[17]
Vancomycin	Caffeine	Rat serum, vitreous, aquaeous humour	Spherisorb 5 ODS 1 (150×4.6 mm, 5 μm)	0.05 mol/L PB[i)], pH 4 and 10% ACN isocratic elution, 25°C	UV 198 nm	8	0.03	0.03–100	[18]

(*Continued*)

Table 5. Continued

Analyte	Internal standard	Matrix	Column[a)]	Mobile phase[b)]	Detector	tR[m)] (min)	LOQ (mg/L)	Lin. range	Ref.
Vancomycin Teicoplanin Daptomycin Colistin	Polymyxin B	Human plasma	Kinetex C18 (50×2.1 mm, 2.6 μm)	A: 0.1% FA in H2O B: 0.1% FA in ACN gradient elution, from 4 to 40°C	MS/MS, ESI (+)	1.62	0.5	0.5–100	[19]
Vancomycin	Vancomycin-des-leucin	Hunam plasma	Acquity UPLC BEH HILIC (100×2.1 mm, 1.7 μm)	A: ACN B: 0.1% FA in H2O gradient elution, 50°C	MS/MS, ESI (+)	2.7	0.3	0.3–100	[20]
Vancomycin Polymyxins (B1,B2,E1,E2)	Dalbavancin	Rat plasma	Jupiter C18 (50×2 mm, 5 μm)	A: 0.1% FA in H2O (pH 2.8) B: 0.1% FA in ACN gradient elution	MS/MS, ESI (+)	1.4	0.001	0.001–5	[21]
Vancomycin Amikacin Gentamicin	Kanamycin B	Human plasma	Hypurity Aquastar (100×2.1 mm, 5 μm)	A: H2O B: ACN C: 0.2 mol/L perfluoropentanoic acid and 0.13 mol/L NH4C2H3O2 buffer in H2O, gradient elution	MS/MS, ESI (+)	3.08	1.0	1–100	[22]
Vancomycin	Vancomycin-glycin	Human serum	Fortis C8 (100×2.1 mm, 3 μm)	A: 0.1% FA in H2O B: 0.1% FA in ACN gradient elution, 40°C	MS/MS, ESI (+)	9.8	none	1.06–84.41	[23]
Vancomycin	none	Human plasma, Microdialisate	Spherimage-80 ODS2 (125×4 mm, 5 μm)	A: MeOH B: 0.025 mol/L K2HPO4, pH 2.75 gradient elution	UV 240 nm	6.5	0.4 plasma	0.4–80	[24]
Vancomycin	none	Rat plasma	Inertsil ODS-3 (100×2.1 mm)	H_2O and ACN (9:1 v/v) with 0.1% FA isocratic elution, 40°C	MS/MS, ESI (+)	4	0.007	0.01–20	[25]
Vancomycin	Norvan comycin	Mouse plasma	Vydac C18 (50×4.6 mm, 3 μm)	A: 0.1% TFAk) B: ACN with 0.1% TFA (95:5 v/v) gradient elution	PDA[j)] 214 nm	8	0.1	0.1–20	[26]
Vancomycin Terbinafine Spironolactone Furosemide and their metabolites	none	Human plasma Urine	Hypersil GOLD C18 (50×2.1 mm, 1.7 μm)	A: 0.1% FA in H2O B: acetonitrile gradient elution, 20°C	UV 215 nm	3	0.11	0.36–20	[28]

(*Continued*)

Table 5. Continued

Analyte	Internal standard	Matrix	Column[a)]	Mobile phase[b)]	Detector	tR[m)] (min)	LOQ (mg/L)	Lin. range	Ref.
Vancomycin	none	Hunam serum	ZIC® HILIC (100×2.1 mm, 5 μm)	A: ACN B: H_2O gradient elution	MS ESI (+)	±15	0.0002	0.0002–0.05	[29]
Vancomycin	none	Human plasma	Nucleosil RP-18 (150×4 mm, 5 μm)	0.005 mol/L KH_2PO_4 (pH 2.8) and ACN (90:10 v/v), isocratic elution, 30°C	UV 229 nm	4.8	0.2	1.0–100	[30]
Vancomycin	Ristocetin	Human serum Hunam plasma	Microsorb-MV Nh2 column (250×4.6 mm, 5 μm)	62% ACN, 14.25% 0.02 mol/L NaH_2PO_4 and 23.75% 0.02 mol/L Na_2HPO_4 buffer, isocratic elution	UV 225 nm	5.5	0.32	5–100	[31]
Vancomycin	Teicoplanin	Dog, rat, primate serum and urine	pre-treatment column: Oasis (50×1 mm, 30 μm) analytical column: Targa C18 (40×2 mm, 3 μm)	A: 0.25% FA in H_2O B: 0.25% FA in ACN gradient elution, extraction column 40°C analytical column 65°C	MS/MS, ESI (+)	1.4	0.001	0.001–10	[32]
Vancomycin	none	Rat plasma	Pre-treatment column: 2x CAPCELLPAK MF Cartridge SCX (10×4 mm, 5 μm) Trap column: CAPCELLPAK MG-C18 (35×4.6 mm, 5 μm) Separation column: CAPCELLPAK MG-C18 (250×4.6 mm, 5 μm)	for pretreatment: 0.067 mol/L PB, pH 5.3 for separation: 0.05 mol/L PB, pH 6.75 and ACN (88:12 v/v) isocratic elution	UV 215 nm	15	0.5	0.5–100	[33]
Vancomycin	Cefaloridine	Human serum	2x pre-treatment column: C18 Corasil (20×4 mm, 35 - 50 μm) analytical column: μBondapack C18 (300×3.9 mm, 10 μm)	for pretreatment: 0.01 mol/L $NH_4C_2H_3O_2$ buffer, pH 3 for separation: mixture of H_2O/ACN/triethyl amine (870:130:4 v/v/v), pH 3 isocratic elution	UV 228 nm	2.16	none	2.0–50	[34]

(*Continued*)

Table 5. Continued

Analyte	Internal standard	Matrix	Column[a)]	Mobile phase[b)]	Detector	tR[m)] (min)	LOQ (mg/L)	Lin. range	Ref.
Free and total Vancomycin	none	Human plasma	first separation system: ASTON C18 (100×4.6 mm, 5 μm) capture column: ASTON SCX (20×4.6 mm, 5 μm) second separation system: ACR C18 (250×4.6 mm, 5 μm)	PUMP1: 0.01mol/L $NH_4C_2H_3O_2$ buffer with ACN, pH 3.8 (90:10 v/v) PUMP2: (Assistant flow Solution) 0.01 mol/L $NH_4C_2H_3O_2$ buffer, pH 3 PUMP3: 0.05mol/L $NH_4C_2H_3O_2$ buffer, pH 5.2 with ACN (82.5:17.5 v/v)	UV 282 nm	8.48	1	0.195–49.92	[35]
Free and total Vancomycin	none	Hunam plasma	Diamonsil C18 (150×4.6 mm, 5 μm)	MeOH and 0.05 mol/L KH_2PO_4 buffer, pH 3.2 (20:80 v/v) isocratic elution	UV 236 nm	10	0.25	0.25 – 50 (free VCM) 0.5 – 100 (total VCM)	[36]
Free and total Vancomycin	Cefuroxime	Hunam serum	Symmetry Shield® RP18 (150×4.6 mm)	A: 0.07 mol/L C2H3NaO2 buffer, pH 5 B: ACN/MeOH (70:30 v/v) gradient elution	PDA 220 nm	6.5	1.6 (total) 0.3 (free)	1.6 – 300 0.3 – 300	[38]
Vancomycin	none	Human serum	SPS octyl-C8 (150×4.6 mm, 5 μm)	0.1 mol/L Na_2HPO_4 buffer (pH 7) and ACN (95:5 v/v) isocratic elution	UV 240 nm	±4	none	0 – 100	[40]
Vancomycin	none	Hunam plasma	Kromasil C18 (250×4.6 mm, 5 μm)	0.025 mol/L Na_2HPO_4 buffer, pH 7 and ACN (88:12 v/v) isocratic elution	ECD[d)]	9.7	1 or 0.5	5 –100 0.5 – 100	[41]

a) The colunm dimension (mm) and particle size (μm) are given in the parenthesis.
b) The column temperature (°C).
c) ACN – acetonitrile.
d) ECD – electrochemical detector.
e) ESI (+) – electrospray ionization in positive ion mode.
f) FA – formic acid.
g) FLD – fluorescence detector.
h) MeOH – methanol.
i) PB – phosphate buffer.
j) PDA – photodiode array detector.
k) TEA – triethylamine buffer.
l) TFA – trifluoracetic acid.
m) tR – retention time.

saline samples (murine bronchoalveolar lavage fluid samples). Other authors used a range of wavelengths from 214 to 240 nm [4,11,14,24,26,28,30,31,33,36,38,40] or 280–282 nm, where the second maximum of absorbance for vancomycin occurs or for the simultaneous determination of other analytes [13, 35]. The UV and PDA detectors are categorized as absorbance detectors that are easy to operate and provide good reproducibility and robustness. These properties enable their widespread integration to routine practices.

However, in comparison with MS detection, UV detection is a less sensitive and specific method that requires better chromatographic separation of the analytes in biological samples and that can require more time for analysis and sample preparation. However, for example, Baranowska et al. [28] developed an UHPLC–UV method for the simultaneous determination of vancomycin, terbinafine, spironolactone, furosemide, and their metabolites (seven analytes altogether) in human plasma and urine, and all of the analytes were separated in 3.3 min. This good separation was achieved with gradient elution and SPE procedures after protein precipitation for the sample preparation. The LOQ of vancomycin of 0.11 mg/L (0.08 μmol/L) is sufficient for clinical practice. The linear range of 0.36–20 mg/L (0.25–13.8 μmol/L) is adequate for clinical practice [28]. However, dilution is required in the case of higher vancomycin concentrations in patient fluids. To the best of our knowledge, the lowest limit of quantification obtained by HPLC–UV was 0.04 mg/L (0.028 μmol/L) in serum [18].

3.2.2 MS detection

Currently, triple quadrupole MS in combination with an ESI source in the positive mode is the most used method for vancomycin detection in biological fluids. This technique enables sensitive determination. MS detection is often connected with easy sample preparation methods such as protein precipitation [16, 17, 19–22] or on-line sample extraction methods [25, 32]. The authors used the predominant doubly protonated molecular ion $[M+2H]^{2+}$ at m/z 725 and the corresponding product ion of m/z 144 for vancomycin monitoring [16, 17, 20–22, 25, 32]. In addition, the application of the mass transitions 725>1306 [23], 724.7>114, and 724.7>99.9 [19] was published. The molecular mass of vancomycin (1449) was not observed in the mass spectrum because the vancomycin molecule was diprotonated [16, 25].

Zhang et al. [16] published an LC–MS method with a hybrid linear ion trap/orbitrap Fourier transform mass spectrometer. The separation of vancomycin (3.75 min) and the atenolol IS (1.94 min) was completed in 5 min with an LOQ of 0.005 mg/L (0.003 μmol/L) and a linear range of 0.05 to 10 mg/L (0.035–6.9 μmol/L). No significant advantages associated to the use of a LTQ/Orbitrap full scan mass spectrometer by comparison with a triple quadrupole spectrometer for vancomycin determination were mentioned. However, in general, the advantage of full mass scan spectrometry is that all the mass spectral information of the sample is collected, which could help with the identification of drugs [16].

To the best of our knowledge, the lowest published LOQ value was 0.0002 mg/L (0.00014 μmol/L) by Sakamoto et al. [29]. The shortest total time of the procedure was 3.3 min (including the time for the autosampler and instrument duty cycles) by Cass et al. [32].

3.2.3 Other detection techniques

Abu-Shandi [8] detected vancomycin in human plasma using fluorescence detection and achieved a lower limit of quantification and better resolution than UV detection. The analysis was carried out at 225 nm as the exciting wavelength and 258 nm as the emission wavelength [8]. An LOQ of 0.005 mg/L (0.003 μmol/L) was achieved, which was significantly lower than the LOQ obtained by UV detection. The linear concentration range was set from 0.005 to 1 mg/L (0.003–0.69 μmol/L), which is too low for real patient samples. In addition, the 27 min analysis of one plasma sample is unfavorable.

Electrochemical detection is another option for vancomycin determination in human plasma. Favetta et al. [41] proposed a method of coulometric electrochemical detection at +700 mV. The linear concentration range was 5–100 mg/L (3.45–69 μmol/L) after 1:80 dilution or 0.5–100 mg/L (0.35–69 μmol/L) after 1:20 dilution. The total analysis time was 12 min.

4 Internal standards (IS)

An appropriate internal standard enables the control of the extraction procedure, LC injection, and ionization variability (in MS detection). The use of this standard is beneficial, especially when multiple sample preparation steps are used (e.g., SPE). For MS methods, the most suitable internal standards are isotope-labeled compounds because of their similar extraction recovery, chromatographic behavior, and ionization response to that of the desired analyte. In addition, isotope-labeled internal standards (e.g., vancomycin deuterated hydrochloride, $C_{66}H_{64}D_{12}Cl_3N_9O_{24}$) enable better compensation of the matrix effect on the ionization of the analyte [20, 23]. However, vancomycin is a bio-product, and its production is hardly possible [23].

König et al. [23] synthesized vancomycin-glycin as a vancomycin derivative. This method of internal standard preparation should be complicated and time consuming for common use, and a methodology has not been yet been developed for routine clinical practice. Moreover, the duration of analysis of 20 min per sample is overly long. However, the authors reported that the method was not optimized for routine clinical use, and this extensive time of analysis was applied to minimize the matrix effect. The retention time of vancomycin was 9.8 min, and the concentration range was 1.06–84.41 mg/L (0.73–58.24 μmol/L). The LOQ for vancomycin was not

evaluated by the authors because it was outside the intended scope of the study [23].

Oyaert et al. [20] used vancomycin-des-leucine (commercially available), which is also almost identical to vancomycin, as an internal standard for the same reason as in the method mentioned above and with the aim to develop a LC–MS/MS method suitable for routine clinical practice. They achieved a sufficient LOQ of 0.3 mg/L (0.21 μmol/L), and the assay was linear in the range of 0.3–100 mg/L (0.21–69 μmol/L). The retention time for vancomycin was 2.7 min, and the total time for the analysis of one sample was 5 min. For the sample preparation, simple protein precipitation with high recovery was used [20]. This method could be considered as a suitable clinical method.

Aminopterin [17], atenolol [16], teicoplanin [32], dalbavancin [21], kanamycin B [22], and polymyxin B [19] were substances also listed as internal standard. Teicoplanin has a similar structure as vancomycin, but in the method used by Cass et al. [32], different retention times were achieved, indicating that there was no possibility of controlling the ionization process in MS detection by using internal standards. Zhang et al. [16] used atenolol, the structure of which is not similar to that of vancomycin, and the retention time was also different. Although, aminopretin is a smaller molecule than vancomycin, similar retention times (2.44 and 2.39 min) were obtained [17]. Kanamycin, dalbavancin, and polymyxin B were used as IS for the simultaneous determination of vancomycin and other drugs. Their structures were similar to the other determined analytes included to the analysis [19, 21, 22].

In UV determination, internal standards are also utilized for sample extraction and LC injection control. The same retention time is not required for the IS and vancomycin as in MS. Large differences in the retention time can lead to the unnecessary extension of the analysis time. This behavior could be observed, for example, in the study by Hagihara et al. [4] in which the retention time of vancomycin in a human serum sample was 8.5 and 13.7 min for the internal standard (caffeine).

Norvancomycin is a structurally close analogue of vancomycin, and a relatively similar retention time was achievedfor both compounds in the study by Muppidy et al. [26]. Other internal standards used in UV detection were e.g., caffeine [4, 18], ristocetin [31], and cefuroxime [38]. However, methods without the inclusion of IS were also validated [13, 14, 24, 25, 28–30, 33, 35, 36, 40, 41].

5 Concluding remarks

Vancomycin belongs to the commonly determined analyte in clinical practice. The most used are immunoassay-based methods. The benefits of these methods are simplicity and low cost in comparison with LC techniques. However, the possibility of cross-reactivity interactions, poor precision and accuracy are their main disadvantages. The application of immunoassays method provides determination of vancomycin levels only in serum and plasma, and there is no possibility of its usage for other human liquids preferred in pharmacokinetic and biodistribution studies.

Disadvantages of immunoassays could be resolved by HPLC-based methods. Several LC methods with UV or MS detection for vancomycin concentration measurements in various biological fluids were mainly introduced in clinical research. In this review, pre-treatment methods and chromatographic conditions for vancomycin determination were summarized and critically compared. Although the modern sample preparation techniques area offers a number of new methods, this overview showed that the conventional protein precipitation with organic solvent is the most used technique with sufficient recovery for vancomycin. Except for the conventional C_{18} fully porous particular technology, core–shell technology as a new trend in column particles was used. In addition, the successful application of HILIC technology was published despite being primarily intended for small molecules.

Although this review focused solely on vancomycin determination, several scientific articles discussed here featured simultaneous determination of vancomycin with other drugs. This trend is showed mainly in new methods and enables the time reduction and is less burdensome for patients with multiple drug therapy.

This article also provides critical discussion in terms of suitability of published methods for routine therapeutic drug monitoring, which could be helpful in practical implementation LC techniques to the routine vancomycin levels monitoring practice or new method development.

New modern chromatographic methods for vancomycin measurements could shorten the time required for sample preparation and analysis and could provide easy approaches for large series of samples.

5.1 Future perspectives

Automated sample preparation with direct sample injection into the LC system could be a possible future trend in vancomycin sample pre-treatment. Reducing the need for manipulation of biological materials leads to the improved safety of laboratory personnel. This is very important because biological fluids from these patients can often contain harmful bacteria (e.g., MRSA). The automation of sample preparation can achieve shorter time requirements. However, method development is highly required, especially for large numbers of samples.

Sample pre-treatment techniques based on microextraction are a modern progressive trend that enables the reduction of sample and solvent volume. In this review, an SPDE method [29] with a custom-made sorbent was mentioned. Another microextraction technique suitable for vancomycin extraction is disposable pipette-tip extraction (DPX). DPX is the miniaturization approach to conventional SPE, where the sorbent is placed loosely between two frits inside a pipette tip. The sorbent is mixed with the sample by turbulent air bubble

mixing. The extraction is performed considerably more frequently than in conventional SPE and is easily automated or semi-automated.

Another promising technique of vancomycin measurement could be founded on the application of sensors. Korposh et al. [42] presented optical fiber long period gratings sensors based on molecular imprinted polymer nanoparticles, which are selective toward vancomycin determination. These sensors were also successfully tested for vancomycin detection in porcine plasma. The measurement provided high sensitivity and selectivity.

In addition, the determination of free vancomycin instead of the total vancomycin concentration is discussed because only the non-protein-bound fraction of vancomycin provides antimicrobial activity.

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