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ADVANCES IN ANALYTICAL METHODS FOR TEICOPLANIN QUANTIFICATION: FOCUS ON HLPC-UV, LC-MS/MS, AND INNOVATIVE SAMPLE PREPARATION STRATEGIES: A COMPREHENSIVE REVIEW

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ABSTRACT

Teicoplanin A glycopeptide antibiotic with selectivity primarily against Gram-positive multi-drug-resistant organisms, including MRSA, poses special analytical problems because of its highly complex mixture of isoforms, high protein and hydrophilic nature. Proper therapeutic drug monitoring (TDM) is also important to achieve both an optimal content and a maximum effect and minimum toxicity, primarily in critically ill patients. This list of review appraises existing and upcoming analytical procedures, and sample preparation strategies to quantify teicoplanin in biological samples HPLC-UV, LC-MS/MS, Fluorescence, and ameliorated sample preparation diligence, including DLLME and MIPs. The HPLC-UV, especially when combined with internal standardization, is validated, low-cost, and workable approach to regular clinical monitoring; however, LC-MS/MS exhibits high sensitivities and selectivity's to be utilized in research applications and special bases. Sample preparation innovations, such as RM-aided DLLME and MIP-assisted extraction serve additionally to make the technique not only selective and recovery but also suitable to overcome the complexity imposed by a matrix and isolate isoforms of interest. As noted in the review, there is a need of a precise, reliable and a practical analytical approach in ensuring that the clinical utilization of teicoplanin can be expanded and effectively fight antimicrobial resistance.

Keywords: Teicoplanin, HPLC-UV, LC-MS/MS, Fluorescence, DLLME, MIPs, molecularly imprinted polymers, Method Validation, Biological Samples

INTRODUCTION

The increasing concern of antimicrobial resistance, particularly in Gram-positive pathogens (i.e. Methicillin-Resistant Staphylococcus aureus (MRSA) is an emerging priority of global attention in terms of public health. Rampant antibiotic therapy has given rise to multi drug resistant organisms especially within the hospital and community acquired infections where more effective treatment and monitoring practices should be devised. Teicoplanin is a glycopeptide antibiotic manufactured by Actinoplanes teichomyceticus that has been used as potential antibiotics in conjuncture with resistant and Gram-positive bacterial infection as it has been reported to possess desirable pharmacokinetics properties, a broad spectrum of antimicrobials, and comparatively lesser toxicity with vancomycin. The antibacterial mechanism of action of doxycycline hydrochloride is by inhibiting cell wall synthesis which is accomplished through binding on the D-alanyl-D-alanine terminus of peptidoglycan precursors preventing the cross-linking of the bacterial cell walls **[1]**.

Teicoplanin is not a single entity but rather a heterogeneous mixture, predominantly composed of five closely related subcomponents (A2-1 to A2-5), with A2-2 being the most abundant. This structural complexity, combined with its high plasma protein binding (approximately 90%), makes therapeutic monitoring particularly challenging. Moreover, teicoplanin exhibits a notably long half-life of 96–100 hours, enabling once-daily administration but necessitating an appropriate loading dose to rapidly achieve therapeutic concentrations. Therapeutic drug monitoring (TDM) is especially important in critically ill patients, where maintaining optimal plasma levels is essential to avoid both subtherapeutic exposure and potential toxicity. Clinically, recommended plasma concentrations are 10–20 µg/mL for standard infections and up to 30 µg/mL for severe or deep-seated infections.

Several analytical methods have been employed for teicoplanin quantification in biological matrices, including fluorescence polarization immunoassay (FPIA), high-performance liquid chromatography with ultraviolet detection (HPLC-UV), and liquid chromatography–tandem mass spectrometry (LC-MS/MS). Among these, LC-MS/MS offers superior sensitivity and specificity, but its high cost restricts its widespread availability in routine laboratories. Conversely, HPLC-UV remains a practical and widely adopted alternative due to its affordability and ease of use. However, both techniques face limitations when applied to teicoplanin, primarily due to its strong protein binding,

complex biological matrices, and the difficulty in separating structurally similar subcomponents. The distribution of sample matrices and analytical techniques used in teicoplanin quantification is illustrated in **Figure 2 and Figure 3**, respectively, highlighting the trends and preferences in current review.

In an effort to address such obstacles, newer sample preparation methods have been evaluated in order to enhance recovery of analysis and lowered sensitivity of methods. DLLME has been noted with simplicity, rapidity and high enrichment factor. Nevertheless, teicoplanin presents a hydrophilic property that limits the ability to extract into a hydrophobic organic phase. In order to increase the efficiency of extraction, reverse micelle -based DLLME has been used, as it has been possible to improve the solubilization of teicoplanin and the transfer of this compound to the extraction solvent. Besides, specifically designed molecularly imprinted polymers (MIPs), especially the boronate affinity, are able to produce certain binding sites that resemble structural and chemical features of teicoplanin. Such water friendly MIPs provide selectivity to the targeted recognition and extraction of teicoplanin mainly when combined with UPLC-MS/MS detection. The resulting technique is able not only to perform sensitive and selective quantification of teicoplanin components but also is able to make the extraction and preconcentration procedure easier and appropriate to carry small clinical and pharmacokinetic studies.

The number of patients subjected to the use of teicoplanin to combat multidrug-resistant infection highlights the necessity of creating precise, trustful, and convenient ways of analysis of the drug in the human plasma. Advanced chromatographic methods and more innovative methods of RM- assisted DLLME and molecularly imprinted solid-phase extraction have a major role to play in meeting the challenges of analysis of teicoplanin due to its complex structure and pharmacological properties **[2, 3]**. The molecular structure of Teicoplanin shown in **Figure 1**.

STRUCTURAL FEATURES OF TEICOPLANIN

STRUCTURE

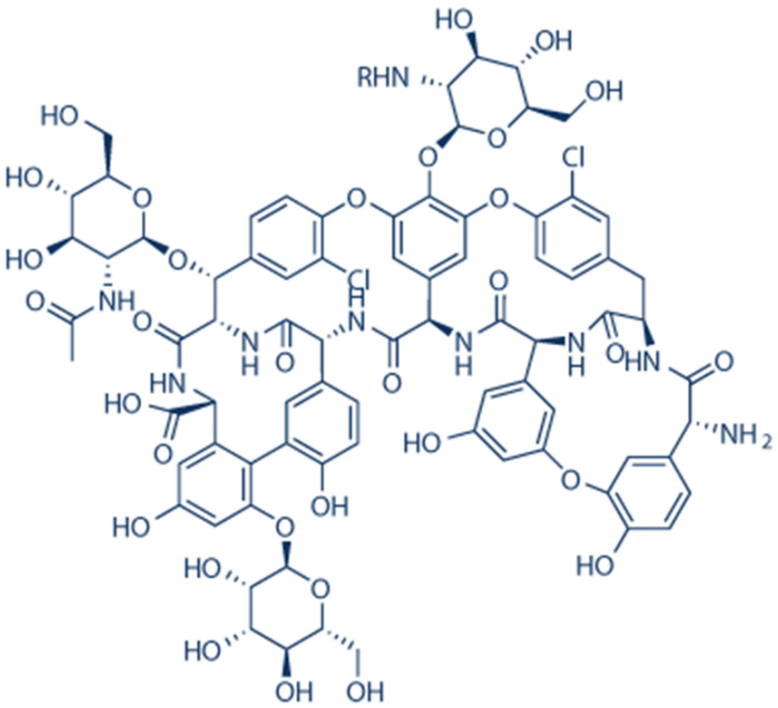

Figure 1: Teicoplanin is a glycopeptide antibiotic characterized by a complex and distinctive molecular structure

- **Core Structure:** Teicoplanin is a cyclic heptapeptide (seven amino acid) backbone that is fused into four rings. Three of these seven amino acids are not protein-encoded or modified (residues based on tyrosine, e.g. 4-hydroxyphenylglycine, 3-chlorotyrosine, 3-chloro-beta-hydroxytyrosine) and 3,5-dihydroxyphenylglycine.
- **Sugar Moieties:** There are three carbohydrate moieties prefixed to this peptide core (D-mannose, N-acetyl-82093229-beta-D-glucosamine and N-acyl-82093229-beta-D-glucosamine).
- **Fatty Acyl Side Chain:** The structures also find diversity in the side chain of fatty acyl, which is attached to one of the sugar residues. The length and conformation of this side chain varies in the major and minor teicoplanin components (A2-1 through A2-5, RS-1 through RS-4).
- **Conformation:** Teicoplanin assumes a bent, concave shape, which enables it to enclose its target peptide into a binding pocket, by wrapping around. N- and C-termiloonirecmp2028 con verge the ligand, and the carbohydrate and side chains give the floor and back of the pocket, and the sugars overhang the binding sit.
- **Molecular Formula:** For the major component teicoplanin A2, the molecular formula is $C_{88}H_{97}Cl_2N_9O_{33}$,

with a molecular weight of approximately 1879.6.

- **Mixture of Compounds:** Commercial teicoplanin is not a single molecule but a mixture of related compounds, all sharing the same glycopeptide core but differing in the fatty acyl side chain. Key Structural Features of Teicoplanin **Table 1**.

Table 1: Key Structural Features of Teicoplanin

FEATURE	DESCRIPTION
Core	Heptapeptide, four-ring cyclized system
Amino Acids	6/7 are modified/non-proteinogenic
Sugars	D-mannose, N-acetylglucosamine, N-acyl glucosamine
Fatty Acyl Side Chain	Variable among the different teicoplanin components
Conformation	Curved, concave, forms a ligand-binding pocket
Molecular Formula (A2)	$C_{88}H_{97}Cl_2N_9O_{33}$
Composition	Mixture of five major and four minor related compounds

ANALYTICAL TECHNIQUES

HPLC-UV

UV-Vis is also part of the analytical techniques employed in quantifiable teicoplanin particularly when partnered with high-performance liquid chromatography (HPLC-UV) **[4, 5]**. Teicoplanin is a multiple isoform glycopeptide antibiotic that shows absorption characteristics at characteristic wavelengths in the UV. Nevertheless, because of the complexity in biological matrices such as human plasma, and the occurrence of various isoforms of teicoplanin (A3-1, A2-1, A2-2, etc.) direct UV-Vis cannot be used to quantitate specifically. Thus, chromatographic separation is combined to UV detection and this is used to isolate the major isoform A2-2 drug that makes up approximately 60 percent of the drug mixture making it specific and minimizing the interference by endogenous materials **[6]**.

Recent articles have been able to optimize an HPLC-UV technique involving the use of an internal standard (polymyxin B) and acidic mobile phase (Na_2HPO_4 buffer and acetonitrile) in an attempt to increase accuracy and precision during determination of teicoplanin in plasma samples. A broad linear range (7.8 to 500 mg/L) spanning below steady-state trough level (10 mg/L) to significantly higher doses that are applicable in critically ill patients is achieved by the method. FDA/ICH guidelines confirmed that validation shows excellent specificity, sensitivity, and accuracy (93.9110.7 percent), and precision (3.7,13.8 percent), as well as favourable recovery and stability during different storage conditions. The internal standard enhances the reliability of quantification as opposed to the previous

methods whereby external standards were used that were characterized majorly by low accuracy and restricted linear range **[5]**.

The usual method of detection is carried out with UV detector at a wavelength of about 220 nm at which teicoplanin has high absorbance because of its chromophore groups **[6]**. This is highly enhanced by the application of an internal standard, e.g. polymyxin B, which makes the results in terms of accuracy and precision of quantification better by accounting variations during samples preparation as well as instrument response. This method has been found to render good linearity at a high concentration range (7.8-500 mg/L) and is clinically significant in the monitoring of teicoplanin level in the critically ill subjects, those who have methicillin-resistant Staphylococcus aureus or Enterococcal faecalis infections **[7]**.

The FDA and ICH method validation has established that the HPLC-UV method is highly specific, sensitive, accurate (93.9-110.7per cent), accurate (3.7-13.8per cent) and recovered at high percentages and is stable across different storage and handling conditions. The chromatograms show that teicoplanin A2-2 and the internal standard are completely separated with no interference and this will guarantee the accurate quantification. In comparison to the LC-MS/MS technique, which is more sensitive and needs costly instruments and specialized skills, HPLC-UV is more convenient and applicable on routine basis in clinics. Furthermore, teicoplanin is isolated in HPLC away from the endogenous and potential degradation products present in plasma thereby overcoming the shortcoming of direct UV-Vis spectroscopy that lacks the specificity in isolation of teicoplanin **[8]**.

In general, HPLC chromatography of teicoplanin is a stable, reproducible and clinically useful procedure that can assist in therapeutic monitoring of teicoplanin by offering accurate measurement of teicoplanin isoforms in plasma. This is by giving the optimal dosing, maximizing therapeutic effect, and minimizing the risks of toxicity in the patient's receiving treatment of serious resistant infections with bacteria. The ability of the method to accommodate different type of samples, as well as its good regulatory compliance, also contributed to the significance of the method in research and even clinical pharmacology **[9]**.

The HPLC-UV method for teicoplanin quantification is a highly optimized and validated analytical approach designed to address the challenges of measuring this glycopeptide antibiotic in human plasma. Teicoplanin exists as a mixture of six main isoforms, with A2-2 being the most abundant and clinically relevant. Direct

UV-Visible spectrophotometry is not suitable for plasma samples because of the complexity of biological matrices and the presence of multiple isoforms, which can cause overlapping signals and interference from endogenous substances. The HPLC-UV method overcomes these issues by first separating the isoforms using a reversed-phase C18 column and an acidic mobile phase composed of sodium dihydrogen phosphate buffer and acetonitrile **[10]**. Detection is performed at 220 nm, a wavelength where teicoplanin exhibits strong absorbance. A key innovation in this method is the use of polymyxin B as an internal standard, which significantly improves the accuracy and precision of quantification compared to earlier methods that relied on external standards. The method demonstrates excellent linearity across a wide range of concentrations (7.8–500 µg/mL), with high accuracy (93.9–110.7%) and precision (3.7–13.8%), and has been validated according to FDA and ICH guidelines. The lower limit of quantification (LLOQ) is 7.81 µg/mL), making it suitable for both low and high therapeutic levels encountered in clinical practice. The method also shows high recovery and stability, ensuring reliable results even with different sample storage and handling conditions. By specifically quantifying the A2-2 isoform and using an internal standard, the HPLC-UV method provides a robust, reproducible, and clinically practical solution for therapeutic drug monitoring of teicoplanin, especially in critically ill patients with resistant infections such as MRSA and Enterococcus faecalis. Compared to more complex and expensive LC-MS/MS methods, HPLC-UV is more accessible for routine clinical use, making it a preferred choice in many hospital laboratories **[11]**.

LC- MS

Another prominent choice is mass spectrometry in combination with liquid chromatography (LC-MS or LC-MS/.MS) as a highly specific and efficient analytical method that can be applied in quantification of teicoplanin in a biological sample (plasma or serum). Teicoplanin is a glycopeptide antibiotic which contains several isoforms (A2-1, A2-2, A2-3, A2-4, and A2-5) and LC-MS techniques allow to detect and quantification of all these components very sensitively and accurately simultaneously.

In the LC-MS/MS methods, teicoplanin is initially isolated chromatographically by LC- the most common methods are the use of reversed-phase C8 or C18 columns with an acidic mobile phase to remove the endogenous compounds present in the plasma and potential interfering agents. Teicoplanin is then determined using the mass spectrometer, which identifies the teicoplanin by its characteristic mass-to-

charge (m/z) ratios and fragmentation patterns, most often through electrospray ionization (ESI) in the positive-ion mode. In the internal ones, internal standards are used to enhance the accuracy of quantification in compensation of matrix effects and instrumental variability (examples of internal standards include Risto cetin, vancomycin hydrochloride or polymyxin B) **[12]**.

The validated LC-MS/MS assays have high linearity across clinically meaningful range of values e.g., 0.2 100 g/mL, low limits of detection and quantitation e.g., LOD ~0.2 0.3 g/mL, LOQ near 1 g/mL, high precision and accuracy and high imprecision is usually less than 7. They demonstrate low levels of ion suppression and matrix effects too, therefore giving a dependable result. Run time of the samples is also fast; usually 3-6 minutes making it possible to analyse multiple samples by high-throughput analysis applicable in the therapeutic drug monitoring (TDM) **[13]**.

Both the Teicoplanin measurements via LC-MS/MS and immunoassays have usually varying degrees of cross-reactivity and consequently differ in their results when it comes to teicoplanin concentration. Immunoassays tend to report higher levels of teicoplanin as compared to LC-MS/MS probably because the latter has higher specificity and reduces the cross-reactivity that immunoassays have. This greater specificity is important in personalized dosing, particularly that of patients in critical care requiring pharmacokinetics to sometimes be unpredictable. LC-MS/MS also fixes both total and free fractions of teicoplanin, which gives more pharmacokinetic understanding **[14]**.

Mass spectrometry-based analysis techniques, LC-MS/MS, in particular, are the gold standard of teicoplanin quantification with regard to sensitivity, specificity, speed and robustness. They become important agents of optimizing the dose regimes based on accurate therapeutic drug monitoring, which eventually achieves better results in patients using teicoplanin **[15-17]**.

DISCUSSION

Teicoplanin is a glycopeptide antibiotic, which contains a substantial complex of isoforms combined with high protein binding and a hydrophilic compound that contributes to significant problems in analyzing this compound. Therapeutic drug monitoring (TDM) will require accurate quantification especially in the case of critically ill patients and in infections that are multi drug resistant to achieve ideal doses and maximize therapeutic and minimize adverse efficacy.

High-performance liquid chromatography with ultraviolet detection can be considered among the more practical and accessible analytical techniques that can be used

routinely in clinical settings **[5]**. The specificity of the method is done by the chromatography separation of isoforms and an internal standard e.g. polymyxin B that increases the accuracy and precision of the method. HPLC-UV has a wide linear response, excellent metrics of validation, regulatory compliance, suitable to clinical practice. It is, however, less sensitive to all the teicoplanin isoforms and is not highly sensitive as other sophisticated methods of mass spectrometry **[7]**.

Teicoplanin cord Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has been considered as the gold standard in quantifying teicoplanin because it is more sensitive and specific than either chromatographic method. The simultaneous detection of multiple isoforms and the high accuracy of the results is especially useful to simple pharmacokinetic studies, and to patients who need precise changes in dosing. Although all these are advantages, the fact that it is expensive and also requires specialized equipment tips the scales on the side of laboratory usage in only specialized or research labs **[13]**.

Teicoplanin extraction and quantification has also been enhanced with innovations in sample preparation techniques (dispersive liquid-liquid microextraction (DLLME) and molecularly imprinted polymers (MIPs)). The hydrophilic nature of teicoplanin is overcome by using DLLME, especially when complemented by reverse micelle methods, which raises extraction efficiency. MIPs, especially those endowed with boronate affinity offer excellent selective extraction and preconcentration, which give rise to precise determination in (multicomponent) biological matrices **[4]**.

Patients with severe infections, particularly the ones aimed against the work of multidrug-resistant organisms, require reliable TDM of Teicoplanin. These clinical needs, laboratory resources available and sensitivity needed should be used to choose the analytical method. The combination of new sample preparation methods with reliable methods of analysis will help to enhance the ease of access to teicoplanin monitoring and their accuracy. In order to achieve optimum therapeutic effects, further designing and standardization of these approaches is necessary to counter continuous antimicrobial resistance **[5]**. The summary of analytical methods foe the estimation of Teicoplanin is presented in **Table 2**. The distribution of sample matrices and analytical techniques used in Teicoplanin quantification is shown in **Figure 1 & 2** respectively.

Table 2: Analytical methods for estimation of Teicoplanin in biological and pharmaceutical sample

S.NO	MATRIX	TECHNIQIES	DETECTION	MOBILE PHASE	COLUMN	FLOW RATE (mL/min)	LINEARITY RANGE (µg/mL)	LOD/LOQ (µg/mL)	REFERENCE
1	Injection	HPLC	UV-278	ACN: MeOH (50:50v/v)	C18	1	70.0-120.0	1.46/4.88	1
2	Plasma	HPLC	UV-265	MeOH: ACN (50:50v/v)	C18	4.5	0.4	NA	2
3	Saliva	HPLC	UV-210	MeOH: H_2O: Ammonium formate: FA	C18	1	NA	1.7/2.3	3
4	Plasma	HPLC	UV-344	FA: MMA-DVB:2,2-azo-bisisobutyronitrile	C18	0.3	1.0	0.1/0.5	4
5	Plasma	HPLC-UV	UV-320	Na_2HPO_4: ACN (78:22v/v)	C18	1.0	7.81-500	2.5/0.1	5
6	Plasma	HPLC-UV	UV-215	Na_2HPO_4: ACN (75:25v/v)	C18	1	3.125	NA	6
7	Plasma	HPLC-UV	UV-210	ACN: Sodium Phosphate monobasic (25:75v/v)	C18	1	NA	NA	7
8	Plasma	RP-HPLC	UV-228	Na_2HPO_4: ACN (75:25v/v)	C18	1	0.8-1.0	0.5/3.0	8
9	Tablet	RP-HPLC	UV-288	MeOH: Ammonium acetate: AA (60:40:0.1v/v)	C18	0.6	1.06-0.89	0.65/1.96	9
10	Tablet	RP-HPLC	UV-254	Buffer: ACN (90:10v/v)	R18	2.3	1.6-2.4	NA	10
11	Serum	UV	UV-240	Sodium tetraborate, sodium dodecyl sulfate, ACN (20:40:11v/v/v)	NA	3	5-55	0.06	11
12	Meat sample	LC MS/MS	MS	MeOH: ACN: Ammonium formate: AA (480:120:0.3:0.04v/v/m/v)	C18	0.5	0.5-50	2.0/5.5	12
13	Plasma	LC MS/MS	MS	MeOH: FA aqueous solution (50:50v/v)	C18	0.5	1-100	0.1/1.3 0.3/4.0	13
14	Serum	LC MS/MS	MS	Ammonium acetate: FA	C18	600	25-75	0.2/1	14
15	Plasma	LC MS/MS	MS	MeOH: ACN: Aqueous zinc sulfate (0.1:80;10v/v/w)	C8	0.3	1.0	1	15
16	Serum	UPLC-MS/MS	HPLC	NA	C18	5.5	NA	NA	16
17	Plasma	Fluorescence	Fluorescence	MeOH: DMSO	NA	NA	60-600	10.84	17

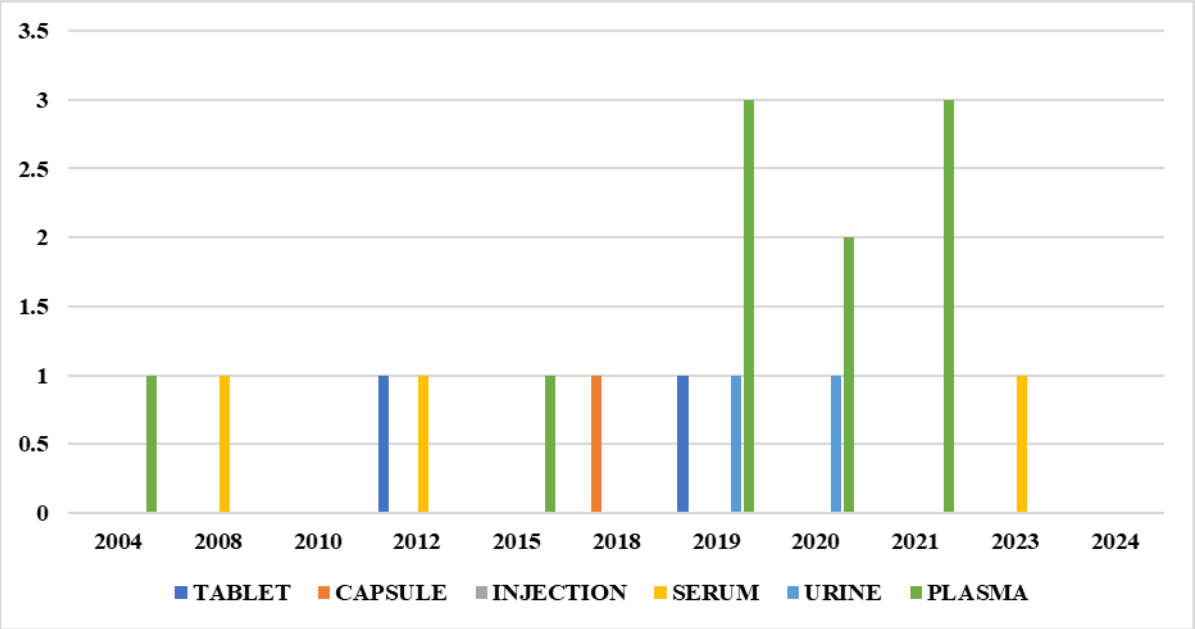

Figure 2: Sample matrices reported for Teicoplanin estimation

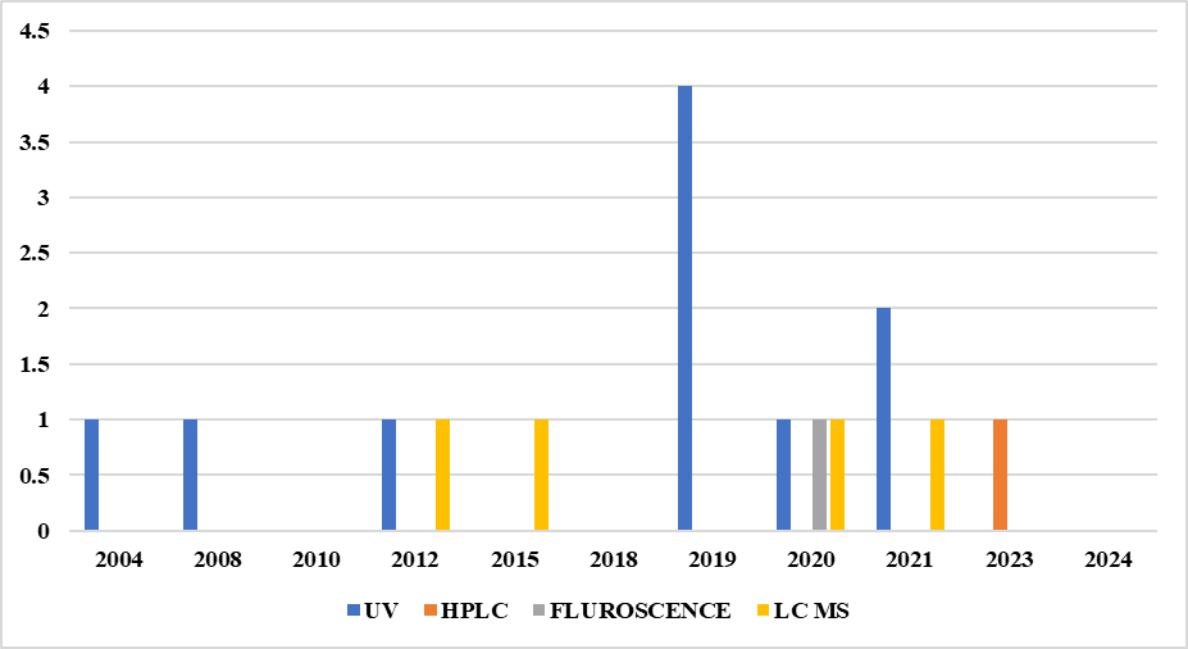

Figure 3: Distribution of analytical methods reported for determination of Teicoplanin

CONCLUSION

An analytical surveillance of Teicoplanin is a rather complicated, yet necessary, element of its clinical use due to the multi-isoform nature of the drug, its highly protein-bound nature, and the necessity of sophisticated therapeutic drug monitoring in hostile infections. Among all the available methods, HPLC-UV, given its reliable protocols, internal standards, specificity, sensitivity, and practical approach, is the most desirable and currently prevailing tool when it comes to normal and day-to-day clinical applications. LC-MS/MS has unsurpassed performance in the analytical world but expense and technical

specifications restrict its utilization to the domain of specialized labs. Most of the recent developments in sample preparation (i.e., RM-assisted DLLME and MIP-based methods) have substantially enhanced both the efficiency and selectivity of the extraction process and made it more convenient and accurate to quantify specific target molecules, even in complex biological matrices. Further development and verification of such methods are essential to optimal dose definitions, decreased toxicities, and eventual more successful patient outcomes in coping with multidrug-resistant diseases by using teicoplanin. Due to teicoplanin's complex isoforms and protein-binding nature, analytical methods like HPLC-UV and LC-MS/MS remain essential, with innovative extraction techniques enhancing accuracy in complex matrices. Further, review or research should be focus on green chemistry analytical method and improve accuracy and precision**.**

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CONFLICT OF INTEREST:

The authors declare that NO conflict of interest among us.

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