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Capillary electrophoretic analysis of the antibiotic vancomycin in innovative microparticles and in commercial formulations

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Abstract

A new fast capillary electrophoretic method has been developed for the analysis of the glycopeptide antibiotic vancomycin in formulations. An electrophoretic run is completed within 3.0 min; fused silica capillaries (100 μm i.d., 8.5 cm effective length and 48.5 cm total length) and a background electrolyte consisting of 12.5 mM, pH 2.5 phosphate buffer are used. The applied voltage is −20.0 kV; samples are injected by pressure (30 mbar × 3 s) at the anodic end of the capillary. The method was successfully applied to innovative controlled release microparticles consisting of a coated albumin core containing vancomycin. A simple procedure has been developed to obtain complete vancomycin extraction from microparticles using a 5% (w/v) sodium dodecyl sulphate aqueous solution. The method has been validated in terms of linearity, precision and accuracy. Good linearity was found in the 0.25–5.00 μg/mL range. Satisfactory precision was obtained, with relative standard deviation values always lower than 3.9%; accuracy was satisfactory, with recovery values between 97.8 and 102.2%. The method is also suitable for vancomycin determination in commercial capsules.

Keywords: Vancomycin; Capillary zone electrophoresis; Glycopeptide antibiotic; Innovative microparticles; Controlled vancomycin release; Commercial capsules

1. Introduction

Vancomycin (Fig. 1) is a glycopeptide antibiotic isolated from the soil microorganism *Streptomyces orientalis* [1], which exhibits bacteriostatic activity against Gram-positive, but not against Gram-negative bacteria, since it is not able to penetrate the external permeability barrier [1–3]. Vancomycin is especially useful in case of penicillin resistance or patient intolerance [4]; it is administered in parenteral form for the treatment of severe Gram-positive infections such as brain abscess, meningitis, peritonitis and for the treatment and prophylaxis of endocarditis. The drug is poorly absorbed from the gastrointestinal tract, therefore it cannot be orally administered to treat systemic diseases; oral administration is thus only indicated in the treatment of local infections, such as serious forms of colitis (e.g. those caused by *Clostridium difficile* or *S. aureus*) [3,4].

In order to optimise vancomycin bioavailability in the treatment of intestinal infections different innovative microparticulate systems have recently been proposed based on pH-dependent, time-dependent or enzyme-dependent release mechanisms [5,6]. In these systems vancomycin is generally dispersed in biocompatible or biodegradable polymeric microparticles able to protect the drug in the gastric environment and to guarantee its release in the colon.

In this view, an innovative formulation consisting of albumin nanospheres coated with stearic acid has been specifically developed to guarantee a pH-dependent release of vancomycin. In these microparticles the inner albumin core containing the drug allows a kinetic control of the release while the coating guarantees drug-core localisation in the intestinal tract. Obviously, the vancomycin content in this innovative formulation has to be determined, especially during the optimisation of the microparticle production procedure, but also for the routine quality control when the laboratory production protocol is already

Abbreviations: BGE, background electrolyte; EOF, electroosmotic flow; LOD, limit of detection; LOQ, limit of quantitation; SDS, sodium dodecyl sulphate

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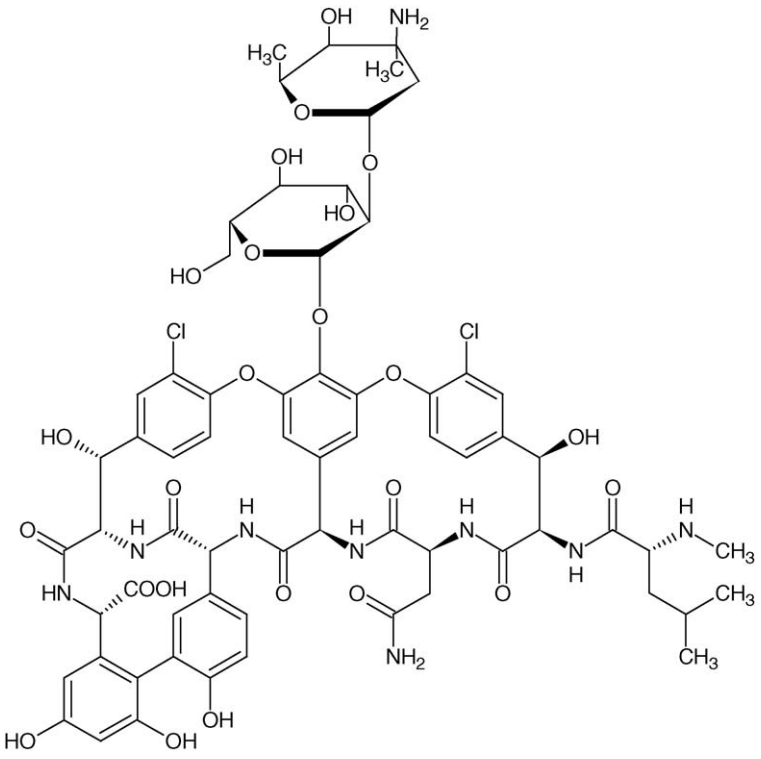

Fig. 1. Chemical structure of vancomycin.

definite. This is a preliminary step needed for the future application of the formulation in vivo, which has not been carried out yet.

Vancomycin is a chiral molecule and is therefore often used for enantiomeric separations; several papers report the use of vancomycin-based stationary phases in HPLC [7] nano-liquid chromatography [8] or capillary electrochromatography [9]. Vancomycin is also used as a chiral selector in capillary electrophoresis [10].

As regards vancomycin determination, HPLC has been widely used, especially in biological samples; some methods have been developed for vancomycin analysis in human or rat serum and plasma using HPLC with UV detection [11,12] or HPLC–UV compared to pyrolysis–mass spectrometry [13], however, HPLC coupled with electrochemical detection has also been used [14]. HPLC has been applied for the determination of vancomycin and degradation or co-fermentation products [15] and to investigate vancomycin stability as a function of pH, buffer concentration and ionic strength [16]. Finally, the official USP XXVIII method for vancomycin analysis uses HPLC with UV detection [17].

With regard to capillary electrophoresis, a CZE method for the analysis of carbapenems reports the possibility of detecting vancomycin as well, but the method has not been developed and validated for this drug [18]. Capillary electrophoresis has also been used for the determination of dimerisation of vancomycin and other glycopeptide antibiotics [19] and to study the conformational stability of vancomycin and other glycopeptides in aqueous solution and their thermal degradation [20]. Micellar electrokinetic chromatography (MEKC) has been used for vancomycin analysis. A MEKC method has been developed for vancomycin determination in human plasma [21] using SDS as a micelle-forming agent. Another MEKC method has been used for the analysis of vancomycin and its impurities in commercial formulations (vancomycin is detected in 6 min) [22], however, the presence of cetyltrimethylammonium chloride in the BGE is detrimental to the sensitivity. Finally, a MEKC method reports the separation of glycopeptide antibiotics (including vancomycin) using SDS micelles [23]. However, the method was not applied to formulations and the migration time for vancomycin was still longer than 8 min. Furthermore, MEKC is surely more complicated than normal CZE.

The method presented herein has been specifically developed for the fast determination of vancomycin in a new innovative formulation and it is more simple and faster than the other methods mentioned, and is also suitable for the rapid quantitative determination of vancomycin in commercial formulations.

2. Experimental

2.1. Chemicals and solutions

Vancomycin hydrochloride ((S_a)-(3*S*,6*R*,7*R*,22*R*,23*S*,26*S*, 36*R*,38a*R*)-44-[[2-*O*-(3-amino-2,3,6-trideoxy-3-*C*-methyl-α-L-*lyxo*-hexopyranosyl)-β-D-glucopyranosyl]oxy]-3-(carbamoylmethyl)-10,19-dichloro-2, 3,4,5,6,7,23,24,25,26,36,37,38,38a-tetradecahydro-7,22,28,30,32-pentahydroxy-6-[(2*R*)-4-methyl-2-(methylamino)]valeramido]-2, 5, 24, 38, 39-pentaoxo-22*H*-8, 11:18, 21-dietheno-23, 36-(iminomethano)-13, 16:31, 35-dimetheno-1*H*, 16*H*-[1, 6, 9]oxadiazacycloexadecino[4, 5-*m*][10,

2,16]benzoxadiazacyclotetracosine-26-carboxylic acid) and stearic acid were purchased from Fluka (Buchs, Switzerland).

All chemicals were analytical grade or better; 85% (w/w) phosphoric acid, methanol, ethanol, acetone and 2 M sodium hydroxide were from Carlo Erba (Milan, Italy), sodium dodecyl sulphate (SDS) was purchased from Fluka (Buchs, Switzerland). Bovine serum albumin, sorbitan monostearate (SPAN 60) and triprolidine hydrochloride (2-[(1E)-1-(4-methylphenyl)-3-(1-pyrrolidinyl)-1-propenyl]-pyridine), used as the Internal Standard (I.S.) for the analysis of commercial formulations, were purchased from Sigma Chemicals (St. Louis, MO, USA); dibenzepine (10-[2-(dimethylamino)ethyl]-5,10-dihydro-5-methyl-11H-dibenzo[b,e][1,4]diazepin-11-one), used as the Internal Standard (I.S.) for the analysis of innovative microparticles formulations was kindly provided by Novartis (Basel, Switzerland).

Ultrapure water (18.2 MΩ cm) was obtained by means of a Millipore (Bedford, MA, USA) MilliQ apparatus.

Stock solutions of vancomycin (1.0 mg/mL) were prepared by dissolving 10.2 mg of vancomycin hydrochloride in 10 mL of water (vancomycin is slightly soluble in alcohol); stock solutions of triprolidine hydrochloride and dibenzepine (1.0 mg/mL) were prepared by dissolving 11.3 and 10.0 mg, respectively, of the pure substance in 10 mL of methanol. Stock solutions were stable for at least 2 months when stored at −20 °C, as assessed by means of spectrophotometric assays.

Standard solutions of vancomycin for the analysis of commercial formulations were prepared fresh every day by diluting stock solutions with water. Standard solutions of vancomycin for the analysis of the innovative microparticle formulations were prepared in water; a suitable amount of SDS was added to the final water solution in order to obtain the same SDS concentration present in the samples.

The background electrolyte (BGE) was a 12.5 mM, pH 2.5 phosphate buffer, prepared as follows: 85 μL of 85% phosphoric acid were dissolved in about 80 mL of water and the solution pH was adjusted to 2.5 with 2 M NaOH. The solution was then transferred into a 100-mL volumetric flask and finally diluted to the mark with water. All BGEs were filtered through a cellulose acetate syringe filter (0.20 μm, Albet-Jacs, Barcelona, Spain) prior to use.

2.2. Apparatus and electrophoretic conditions

All experiments were carried out using an Agilent (Waldbronn, Germany) ^{3D}CE apparatus equipped with a diode array UV detector (DAD). Analyses were carried out on an uncoated fused silica capillary (Composite Metal Services Ltd., Hallow, UK; 48.5 cm total length, 8.5 cm effective length, 100 μm i.d., 375 μm o.d.). The detection wavelength was 210 nm. A constant voltage of −20.0 kV was applied with the capillary thermostatted at 25.0 °C. Samples were injected by pressure (30 mbar × 3 s) at the anodic end of the capillary.

Before use, new capillaries were rinsed with water (5 min), 1 M NaOH (5 min) and water again (10 min). Before each run, the capillary was conditioned with the BGE for 1 min, applying high pressure (3 bar) at the anodic end of the capillary; after the electrophoretic run the capillary was rinsed with water for 2 min applying high pressure (3 bar) at the anodic end.

At the end of each working day the capillary was rinsed with water for 5 min, 1 M NaOH for 5 min and water again for 10 min.

2.3. Innovative microparticle preparation

Microparticles were prepared by solubilising 3 g of albumin in 100 mL of water; about 120 mL of acetone were added to this solution in order to coacervate the albumin. Acetone was evaporated by heating the coacervate at 100 °C thus obtaining cross-linked albumin nanospheres. The suspension was finally dried using a spray-drying apparatus (Büchi Mini Spray Dryer, B-191, Flawil, Switzerland) with an inlet temperature of 105 °C and an outlet temperature of 55 °C.

An aliquot of 50 mg of the microparticles was then transferred to 25 mL of an aqueous vancomycin solution (1 mg/mL) and left under agitation for 24 h. After that, loaded microparticles were separated from the remaining solution by ultracentrifugation (12,000 rpm, 30 min, 4 °C); microparticles were then washed with water, immediately ultracentrifugated again and freeze-dried.

Microparticle coating was carried out as follows: 300 mg of microparticles were transferred into a beaker containing 50 mL of an ethanolic solution of 250 mg of stearic acid and 50 mg of SPAN 60; the suspension obtained was kept under agitation, nebulised and dried using a spray-drying apparatus (inlet temperature 50 °C, outlet temperature 25 °C).

2.4. Innovative microparticle analysis

An amount of 3.0 mg of microparticles was weighed and transferred into a 3-mL volumetric flask and brought to volume with a 5% aqueous solution of SDS (w/v). The suspension thus obtained was sonicated for 15 min, left at room temperature for 20 min and then centrifuged at 3400 × *g* for 15 min. The supernatant was finally diluted in water and analysed by capillary electrophoresis.

2.5. Commercial capsule analysis

Capsules of Farmaciclin® (Farma Uno S.r.l., Salerno, Italy) with a declared content of 256 mg of vancomycin hydrochloride, corresponding to 250 mg of vancomycin free base and polyethylenglycol 6000 as excipient, were analysed.

The content of 20 capsules was accurately weighed, then ground to a fine powder and mixed in mortar. An amount equivalent to 10 mg (declared) of vancomycin free base was weighed and transferred into a 10-mL volumetric flask, and about 8 mL of ultrapure water were added. The solution was then sonicated for 10 min and finally brought to volume with water.

This solution was further diluted with water and analysed by capillary electrophoresis.

2.6. Method validation

2.6.1. Calibration curves

Five-point calibration curves were set up in the 0.25–5.00 μg/mL concentration range by plotting the following quantity: $(\text{area}_{\text{vancomycin}}/t_{\text{m vancomycin}})/(\text{area}_{\text{IS}}/t_{\text{m IS}})$ against the corresponding concentration of vancomycin expressed as micrograms per millilitre. Calibration curves were obtained by means of the least-square method.

2.6.2. Determination of vancomycin content in commercial capsules

The solutions obtained from commercial capsules of vancomycin were analysed by CZE, after dilution in water at the nominal concentrations of 0.50, 2.00 and 5.00 μg/mL; the values of $(\text{area}_{\text{vancomycin}}/t_{\text{m vancomycin}})/(\text{area}_{\text{IS}}/t_{\text{m IS}})$ were interpolated on the calibration curve, comparing the concentrations obtained to those declared by the manufacturer.

2.6.3. Determination of vancomycin content in innovative microparticles

The solutions obtained from the extraction of vancomycin from microparticles as described in Section 2.4 were analysed after 1:100 or 1:200 dilution and the values of $(\text{area}_{\text{vancomycin}}/t_{\text{m vancomycin}})/(\text{area}_{\text{IS}}/t_{\text{m IS}})$ were interpolated on the corresponding calibration curve, in order to determine the content of vancomycin in the formulation. The vancomycin percentage in the microparticles was finally calculated using the formula:

$$\frac{\text{Vancomycin concentration}}{\text{microparticle concentration}} \times 100$$

2.6.4. Precision assays

Precision was assessed on both standard solutions and on innovative and commercial formulations according to USP XXVIII requirements [24]. Vancomycin was extracted from commercial capsules and diluted at three different nominal concentration levels: 0.50, 2.00 and 5.00 μg/mL (for standard solutions, vancomycin was simply diluted to the same concentrations).

Each assay was repeated six times within the same day and six times over different days to calculate repeatability values and intermediate precision values, respectively, expressed as percentage relative standard deviations (R.S.D.%).

The quantitation limit (LOQ) and the detection limit (LOD) were determined following the USP XXVIII guidelines [24].

2.6.5. Accuracy

In order to evaluate the accuracy of the method, a known amount of vancomycin hydrochloride powder was added to a known amount of the powder content of the capsules or of the microparticles, to obtain three different levels of spiking corresponding to 0.50, 1.00 and 2.00 μg/mL. The samples were analysed and the mean recovery calculated. In order to evaluate repeatability, each assay was carried out six times for each concentration added.

3. Results and discussion

3.1. CZE conditions

Vancomycin is a glycopeptide antibiotic with quite a complex structure; it has in fact several acid and basic functional groups, and therefore, its net charge in solution strongly depends on the solution pH. Vancomycin pK_a values are: 2.9, 7.2, 8.6, 9.6, 10.4 and 11.7 with 7.2 being the isoelectric point [25]. Consequently, vancomycin can be positively or negatively charged depending on the pH of the BGE, and will migrate to the cathode or to the anode, respectively. Considering the presence of the electroosmotic flow (EOF) and the limited mobility of vancomycin, the analyte will always migrate towards the cathode, but its apparent mobility will be higher than the EOF marker mobility at acidic pH, and lower at basic pH (considering that vancomycin's isoelectric point is at neutral pH). Several BGE pH values were investigated; at first a 50 mM pH 8.0 phosphate buffer was tested, following the literature [18]: vancomycin showed an asymmetric peak, very close to the EOF (the voltage was −15.0 kV). In order to increase the ionisation percentage of vancomycin and thus to better separate the vancomycin peak from the EOF, the pH was increased to 9.0 (borate buffer) and then to 10.0 (carbonate buffer). The separation between the vancomycin peak and that of the EOF increased in both cases, however, the analyte peak was still quite asymmetric.

Buffers at acidic pH values were then investigated: pH 2.5 phosphate, pH 3.5 formate, pH 4.5 citrate and pH 5.5 acetate. At low pH values, vancomycin ionisation percentage increases, while the EOF influence decreases. The pH 2.5 phosphate buffer gave the best results in terms of peak efficiency and migration times and was therefore chosen for further investigations.

To obtain a short analysis time, the voltage was increased to −20.0 kV, while the BGE used had to be diluted to 12.5 mM in order to maintain an acceptable current value. The voltage was not further increased because it would have also been necessary to further dilute the BGE (to keep acceptable current values) and this would have resulted in a loss of buffering power.

3.2. Method validation for commercial capsules

3.2.1. Standard solutions

Triprolidine was chosen as the I.S., because it is positively charged at acidic pH values and migrates faster than vancomycin, thus not influencing the total run time. The analyte injection from the short end of the capillary (effective length = 8.5 cm) allows to obtain short analysis times: an entire electrophoretic run lasts less than 3.0 min. Migration times for vancomycin and the I.S. are 2.6 and 0.9 min, respectively. Good linearity was found in the vancomycin concentration range from 0.25 to 5.00 μg/mL: the calibration plot was obtained reporting the value $(\text{area}_{\text{vancomycin}}/t_{\text{m vancomycin}})/(\text{area}_{\text{IS}}/t_{\text{m IS}})$ against the corresponding concentration of vancomycin expressed as micrograms per millilitre. The calibration equation was $y = 0.01076 + 0.38794\,x$ ($r^2 = 0.9996$). The LOQ value was 0.25 μg/mL and the LOD value was 0.10 μg/mL.

To validate the method the repeatability and the intermediate precision were calculated at three different vancomycin concentration values, repeating each assay six times. Satisfactory results were obtained: the mean R.S.D.% value was 1.1% for repeatability and 1.8% for intermediate precision.

3.2.2. Commercial capsule analysis

The method was applied to the analysis of oral capsules containing vancomycin (Farmaciclin®). As both vancomycin and the excipient are very soluble in water, stock solutions were directly prepared dissolving the capsule powder content in water; as for standard solutions of vancomycin, further dilutions were made in water and the samples injected and analysed.

The electropherograms obtained (Fig. 2a) show the same appearance as those obtained analysing standard vancomycin solutions; no interference from the matrix can be observed.

The amount of drug found of declared was calculated at three different nominal vancomycin concentration values. The mean value of vancomycin found of declared corresponds to 99.1%. This value is in the 90.0–115.0% range indicated in USP XXVIII [17] for vancomycin capsules. In order to calculate repeatability and intermediate precision each assay was repeated six times within the same day and in different days, respectively. The satisfactory results obtained are reported in Table 1.

Accuracy was also evaluated and the main recoveries obtained were between 99.0 and 100.5%. The corresponding repeatability and intermediate precision values are reported in Table 2. The satisfactory results obtained confirm that the method can be applied to the analysis of vancomycin in commercial capsules.

3.3. Method validation for innovative microparticles

The method was modified in order to apply it to the analysis of innovative microparticles containing vancomycin.

Since the extraction procedure adopted for commercial capsules was not suitable for this formulation, it was necessary to develop a new procedure.

In fact, the coated albumin matrix of this formulation has very different chemical–physical properties with respect to the capsule matrix. At first, extraction of vancomycin was carried out using pure methanol as a solvent, trying to dissolve the microparticle coating and to denature the albumin core: in this case, extraction was not complete, probably due to the low solubility of vancomycin in alcohols. As a second try, the microparticles were treated with water, which is a good solvent for the analyte; in this case as well a single extraction was not sufficient to remove all vancomycin from the microparticles.

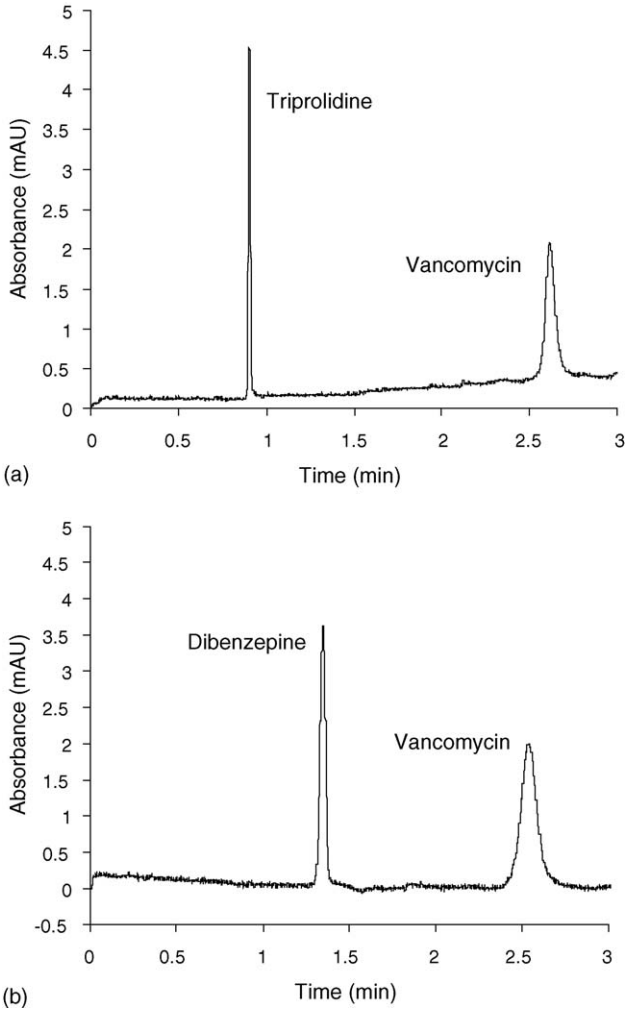

Fig. 2. (a) Electropherogram obtained from the analysis of Farmaciclin® commercial capsules (nominal vancomycin concentration 2.00 μg/mL) containing the I.S. triprolidine (2.00 μg/mL); (b) electropherogram of innovative microparticle solution diluted 1:100 from the stock (microparticle nominal concentration 10.00 μg/mL) containing the I.S. dibenzepine (2.00 μg/mL). CZE conditions — BGE: 12.5 mM, pH 2.5 phosphate buffer; capillary: 8.5 cm effective length; voltage: −20.0 kV; detection wavelength: 210 nm; temperature: 25.0 °C; injection: pressure, 30 mbar × 3 s.

Finally, the extraction was carried out using an aqueous solution of SDS (5%, w/v): this solvent gave the best results and allowed to obtain complete vancomycin extraction. SDS was chosen because it denatures proteins (i.e., albumin which constitutes the microparticle core), helps to solubilise the lipophilic microparticle coating (i.e., stearic acid and SPAN 60) and is compatible with CZE and with vancomycin (in fact some papers report MEKC analytical methods for vancomycin [21–23]). Van-

Table 1
Amount of vancomycin found of declared in commercial capsules (Farmaciclin®)

Vancomycin nominal concentration (μg/mL)	Amount found of declared, %	Repeatability R.S.D.%[a]	Intermediate precision R.S.D.%[a]
0.50	99.8	1.8	3.7
2.00	98.9	0.9	2.8
5.00	98.6	0.8	1.3

[a] $n = 6$.

Table 2
Accuracy of the method

Matrix	Vancomycin amount added (μg/mL)	Mean recovery, %[a]	Repeatability, R.S.D.%[a]	Intermediate precision, R.S.D.%[a]
Commercial capsules	0.5	100.5	1.4	2.0
	1.0	99.0	1.2	1.8
	2.0	100.4	1.1	1.5
Innovative microparticles	0.5	98.6	–	3.7
	1.0	97.8	–	3.5
	2.0	102.2	–	2.9

[a] $n = 6$.

comycin turned out to be stable in 5% SDS solution: in fact, the electrophoretic peak of vancomycin in the presence of SDS has the same appearance, migration time and symmetry coefficient of those obtained in the absence of SDS. Moreover, the UV spectra of vancomycin obtained with the CE-DAD apparatus have the same shape in both cases. In fact, the low concentration of SDS in the sample, and the fact that the only source of SDS is the injected sample, mean that it has no effect whatsoever on the migration of the analyte.

To verify that vancomycin extraction was complete under these conditions, a second extraction was carried out on the powder remaining after centrifugation. This solution was treated and diluted exactly in the same way as in the first extraction procedure; CZE analysis showed no vancomycin peak (i.e., a vancomycin concentration lower than the LOD value).

The extraction procedure was also carried out using a more diluted aqueous SDS solution (0.5%), but in this case it was not possible to obtain a complete vancomycin extraction in a single step. For these reasons a 5% aqueous SDS solution was chosen as the best solvent for the microparticles.

3.3.1. Standard solutions

When standard solutions of vancomycin were used for the analysis of innovative microparticle formulations, a known amount of SDS was added to the samples, in order to obtain the same SDS concentration as in the microparticle samples.

Using SDS, however, led to stability problems with the internal standard triprolidine; this molecule, in fact, resulted to be unstable in this SDS solution. Consequently, the internal standard had to be changed: dibenzepine turned out to be the best choice, because it is stable in the presence of SDS.

In the electropherogram, the peak corresponding to dibenzepine (migration time 1.3 min) is baseline separated from the vancomycin peak and the total run time is not influenced. The presence of SDS in the samples does not change migration times or the appearance of the vancomycin peak.

The method thus modified was validated. Good linearity was found in the range from 0.25 to 5.00 μg/mL: the calibration plot was obtained reporting the value $(\text{area}_{\text{vancomycin}}/t_{\text{m vancomycin}})/(\text{area}_{\text{IS}}/t_{\text{m IS}})$ against the corresponding concentration of vancomycin expressed as micrograms per millilitre. The calibration equation was $y = 0.00273 + 0.37914\,x$ ($r^2 = 0.9998$). Repeatability was calculated at three different concentration values repeating each assay six times in the same day and in different days. The mean R.S.D.% value obtained is 2.8% for repeatability and 3.2% for intermediate precision. The LOQ and LOD values of vancomycin were 0.25 and 0.10 μg/mL, respectively.

3.3.2. Analysis of innovative microparticles containing vancomycin

The microparticle solution containing SDS, obtained as described in Section 2.4, was diluted in water and analysed. The electropherogram obtained is shown in Fig. 2b.

Comparing the electropherogram to those obtained analysing standard vancomycin solutions, no differences can be observed as regards retention times and peak shapes.

The amount of vancomycin loaded into the microparticles was evaluated after diluting 1:100 and 1:200 the stock solution (the microparticle concentrations correspond to 10.0 and 5.0 μg/mL, respectively). The vancomycin amounts found were 19.9% (w/w) for 10.0 μg/mL of microparticles and 19.7% (w/w) for 5.0 μg/mL of microparticles; the intermediate precision was also evaluated and resulted satisfactory: R.S.D. values of 3.9 and 3.7% were obtained for 10.0 and 5.0 μg/mL of microparticles, respectively.

Accuracy was calculated on three different spiking concentration levels (each assay was repeated six times) obtaining satisfactory results for recovery values and intermediate precision (Table 2).

It should be pointed out that repeatability was not evaluated on innovative microparticles because the time needed for a complete analytical procedure (vancomycin extraction, samples preparation and CE analysis) does not allow to repeat it six times within a single day.

4. Conclusions

A new and very simple method based on CZE has been developed and successfully applied to the analysis of vancomycin in an innovative microparticle formulation. The method allows to carry out the analyses within short run times (less than 3.0 min) using a very simple background electrolyte (phosphate buffer). Furthermore, no BGE additive is necessary such as SDS or other micelle-forming agents needed for reported MEKC methods, which also require longer analysis times.

Furthermore, the use of CZE allows to avoid the need for the high amounts of expensive and toxic organic solvents used in HPLC methods.

The method has been validated with satisfactory results in terms of precision and accuracy; the sensitivity obtained (LOD = 0.10 μg/mL) is suitable for the purpose of the present work.

Complete vancomycin extraction is obtained by means of a simple procedure; no interference from the matrix is observed in the CZE analysis of the innovative formulations. The method is therefore suitable for a very fast vancomycin determination in innovative microparticles. Moreover, the proposed method can also be used for the quality control of commercial capsules containing vancomycin.

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