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Determination of linezolid and its achiral impurities using sweeping preconcentration by micellar capillary electrophoresis

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Abstract

Linezolid is the first compound of a truly new class of antibiotics—the oxazolidinones. The elaborated method of capillary electrophoresis (CE) of linezolid separation from its achiral impurities was successfully performed using sweeping preconcentration, followed by UV absorption detection at 254 nm. The best results were obtained with 125 mM Tris buffer, pH 2.0, with addition of 20% (v/v) methanol as background electrolyte. Sodium dodecyl sulfate (150 mM) was added to the electrolyte in the inlet vial as the sweeping agent. The separation was carried out at negative polarity. Then, the optimized method was validated in terms of linearity, accuracy and precision. Sweeping preconcentration of linezolid provides detection limit at 0.05 μg/ml level.

The evaluated CE method was applied in the analysis of medicinal product containing linezolid–linezolid solution for infusion.

Keywords: Pharmaceutical analysis; Linezolid; Achiral purity; Antibiotics

1. Introduction

Linezolid (Fig. 1) the first available oxazolidinone antibacterial agent, shows strong activity against Gram-positive pathogens, including multidrug-resistant organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA), multidrug-resistant *Streptococcus pneumoniae*, vancomycin-intermediated *Staphylococcus aureus* and vancomycin-resistant *Enterococcus spp*. strains.

Linezolid is currently the only antibacterial agent which can be administered orally (as well as intravenously) with strong activity against MRSA. It may be particularly useful as an alternative to vancomycin, in patients whose renal function is impaired, in cases of patients with poor or lack of intravenous access and in patients who require outpatient therapy, or who do not tolerate glycopeptides [1–4].

The UV photometric detector is the most widely used detector in CE. One of the main disadvantage of this common detection technique, particularly when compared to liquid chromatographic techniques, is the poor concentration sensitivity (10^{-5} to 10^{-6} mol/l) resulting from minute injection volumes (typically <1% capillary length) needed to maintain high efficiency and a short optical pathlength equal to the capillary diameter. Several strategies have been proposed to improve detection limits in CE system with UV detector, including, special equipment (e.g. Z-shaped, multi-reflection and bubble cell), and sample preparation methods (e.g., liquid–liquid or solid-phase extraction). The main drawback of these arrangements is the danger of losing separation resolution and time consuming procedures. Therefore efforts were undertaken to establish simplest and reliable techniques, not requiring complicated procedures or/and special equipments.

Various on-line preconcentration techniques have been developed over the past 15 years and there are now several approaches capable of providing 10–100 000-fold increase in sensitivity. Among them are: sample stacking [5–12], sweeping [13–18] (performed individually or in combination) transient isotachophoresis [19–21] and dynamic pH junction [22]. These methods require modification of CE system and rely on a distinct focusing mechanism based on different electrolyte properties between sample and background electrolyte (BGE) zones, such as conductivity (ionic strength), additive concentration

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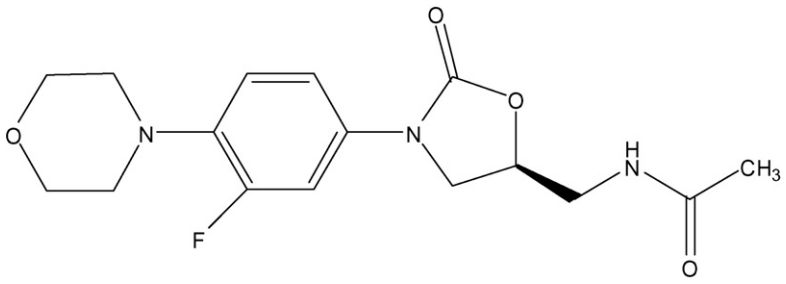

Fig. 1. Chemical structures of linezolid.

(analyte–additive interactions), electrolyte co-ion mobility and buffer pH, respectively. In general, these techniques are designed to compress long analyte bands within the capillary, thereby increasing the volume of sample which can be injected without loss of CE efficiency. The most popular among mentioned techniques are: stacking and sweeping.

The first one is based on the electric field strength differences between the sample zone and the BGE. In brief, sample stacking occurs at the boundary between the high electric field sample zone and low electric field BGE zone. It results from a rapid change in migration velocity of micelles (carrying neutral or ionic analytes) when passing from one region to another.

The second, on-line preconcentration technique sweeping, was initially demonstrated by Quirino and Terabe in 1999 to describe a new approach for the preconcentration of neutral analytes in micellar electrokinetic capillary chromatography (MEKC) through association with a surfactant [13–15]. It is defined as analyte particles picking and accumulating by the pseudostationary phase (PS) that penetrates the sample region devoid of the PS. Unlike stacking, this process is theoretically independent of the BGE and sample zone conductivities. The only requirement for sweeping to take place is the absence of the PS in the sample matrix.

Improvements in these approaches and slight variations have quite often been given different and distinct names which have led to further confusion. Summary of various on-line preconcentration techniques has been described recently, by Breadmore [23], where some attempts have been made to consolidate these approaches based on the principles of the preconcentration mechanism.

On-line concentration techniques have been applied mostly in drug analysis of biological fluids [24–26], but also in drug purity method development, it is indispensable for reducing limits of detection (LOD) or increasing concentration sensitivity [27].

Several methods for linezolid determination in bulk material and human serum were published. Method using high-performance liquid chromatography (HPLC) has been reported for determining linezolid in bulk material [28]. At this paper two unknown impurities were isolated and characterized. Scholl et al. [29] used MEKC for determination of linezolid and some of its congeners. However, the aim of that method was not separation linezolid from its known achiral impurities. Kitahashi and Furuta [30,31] described method for determination of linezolid in human serum using MEKC.

The major aim of this research was to elaborate simplest and reliable method for determination of linezolid and its achiral impurities (fourteen) and then use evaluated method for analysis of medical product containing linezolid.

Separation of linezolid and its chiral impurity—*R*-linezolid using heptakis-(2,3-diacetyl-6-sulfato)-beta-cyclodextrin as chiral selector have been already conducted in our laboratory and the results were presented [32].

Separation and identification of impurities are very important to fulfill the requirements of Government Agency deal with monitoring and control of quality and safety of the drugs.

2. Experimental

2.1. Apparatus

Capillary electrophoresis experiments were carried out on Quanta 4000E CE system (Waters, Milford, MA, USA) equipped with 30 kV power supply and UV spectrophotometric detector, connected to data collection system suitable to perform both hydrodynamic and voltage injection. The selected detection wavelength, using a mercury lamp with appropriate filter, was 254 nm. Separations were performed in an uncoated fused-silica capillary: [50 cm (effective length 42 cm) × 75 μm i.d.] (Waters, Milford, MA, USA), which was operated at 15 kV in reversed polarity (− → +) thermoregulated at 25 °C, and hydrodynamic injections ranging from 10 to 120 s were applied.

Peak Purity Measurements were performed on Agilent HP3D Capillary Electrophoresis System (Waldbronn, Germany) equipped with photodiode array detector (DAD) and software 3D-CE Chemstation rev A.10.02.

The HPLC experiments were carried out on a chromatograph series LC-10Avp Shimadzu (Kyoto, Japan) with UV detector set at 254 nm. Separation was achieved on a Prodigy 5 μ ODS3 100A (250 mm × 4.0 mm i.d.) (Phenomenex, Torrance, CA, USA).

HPLC assay was performed according to producer (Pfizer, Inc.), validated method.

2.2. Standard and reagents

Chemicals of analytical-reagent grade were used. BGE solutions were prepared using TRIS (hydroxymethyl)-aminomethane from Merck (Darmstadt, Germany), hydrochloric acid from POCH (Gliwice, Poland), methanol from Lab-Scan

analytical sciences (Dublin, Ireland) and sodium lauryl sulfate (SDS) from AppliChem (Darmstadt, Germany). Acetonitryle (ACN) from Lab-Scan analytical sciences (Dublin, Ireland), ortho-phosphoric acid 85% from Fluka (Steinheim, Switzerland), sodium hydroxide from POCH (Gliwice, Poland). Deionized (D.I.) water was obtained from a Labconco System (Millipore, Bedford, MA, USA).

Linezolid Resolution Solution (LRS) and standard of linezolid were obtained from Pharmacia Corporation Reference Standard. Linezolid, solution for infusion 2 mg/ml (containing: linezolid, glucose monohydrate, sodium citrate, citric acid anhydrous, hydrochloric acid or sodium hydroxide, water for injections) were obtained from Pfizer (Groton, CT, USA).

2.3. Solutions

BGE, final concentration of 125 mM TRIS, was adjusted to pH 2.0 with concentrated HCl, then 20% (v/v) methanol was added to buffer. SDS at concentration of 150 mM, acting as a sweeping agent, was dissolved in BGE (BGE + SDS). BGE (without SDS) was used for rinsing each run prior to injection and in the outlet vial. BGE with 150 mM SDS was applied in the inlet vial.

The fundamental condition for sweeping is an absence of pseudophase in the sample; hence studied compounds solution was prepared in diluted buffer (BGE-1:5) without SDS.

For the long hydrodynamic injection, the samples in water cannot be used because of water very high electric resistance, therefore 5 times dilute BGE without SDS was applied as a diluent (dilute buffer solution) for all solutions preparations in CE experiments.

Linezolid solution for infusion (2 mg/ml) was diluted to about 0.32 mg/ml with dilute buffer solution.

The procedure for linezolid assay and its related compounds were as follows: test solution (linezolid solution for infusion) and standard of linezolid at concentration around 0.32 mg/ml were injected (120 s) and measured at 254 nm.

2.4. Procedures

BGE (without SDS) was prepared daily, filtered through a 0.45 μm HV Durapore membrane filters from Millipore (Ireland) assembled in vacuum pump and then degassed. After this procedure 150 mM of SDS was added to the BGE (as inlet vial).

The capillary was conditioned every day with methanol for 10 min, then rinsed with deionized water for 5 min, followed by rising with 0.1 M NaOH for 10 min, then with deionized water for 5 min and finally with the BGE (without SDS) for 10 min.

Between each measurement, the capillary was rinsed with BGE (without SDS) for 2 min prior to injection. A large sample plug (devoid of micelle), was injected into the capillary which was previously filled with a buffer not containing micelles. After injection the capillary inlet was placed into BGE + SDS and the voltage of reverse polarity mode was applied.

Linezolid Resolution Solution (LRS)—a mixture of linezolid and its achiral impurities, according to manufacturer specification consists of process impurities, degradation products and synthetic precursor, marked: A–N.

Linezolid Resolution Solution and samples of preparation were introduced to the capillary during various time periods. Injection times of 10, 20, 30, 40, 50, 70, 90, 120 s approximately correspond to 3.43, 6.86, 10.29, 13.72, 17.15, 24.00, 30.87, 41.16 cm of capillary length, respectively.

3. Results and discussion

In the present study an attempt was made to elaborate CE method for separation and determination of linezolid and its achiral impurities using sweeping preconcentration techniques by MEKC.

Several screening experiments were performed to study the influence of pH on behavior of linezolid in CE. Results of the experiments indicate that, linezolid is unionized in background electrolyte (BGE) above pH 4, while under pH 4 linezolid is protonated.

3.1. Optimization

In general, two alternative approaches of sweeping are possible: with reverse migrating micelles (RM-Sweep) and with normal migration micelles (NM-Sweep).

Sweeping have one major limitation, the extent of the swept zone is governed by the interaction of each individual analyte with the pseudophase, according to equation presented in Quirino and Terabe paper [13]. According to equation much better focusing can be achieved in case of analytes that interact more strongly with the pseudophase. It also was emphasized that the retention factor plays the major role in the narrowing of ionic analyte zones and the applicability of sweeping for ionic analytes having great affinities toward the charged pseudostationary phase. The choice of RM-Sweep technique to separate linezolid from its achiral impurities was made after consideration a known advantage of low EOF over high EOF techniques [33] and situation that at low pH linezolid is protonated, while SDS is anionic.

When the BGE is kept at a low pH it causes EOF reduction, therefore, reversed polarity is needed in order to enable migration of micelles (SDS) in proper direction towards the detector. When large sample plug (devoid of micelle) was injected into the capillary, which was previously filled with buffer not containing micelles, then the capillary inlet was placed into an anionic micellar BGE solution and the voltage of reverse polarity mode was applied. In the meantime as the micelles migrated towards the detector they swept the analytes. Under these experimental conditions, positively charged linezolid migrated towards incoming zone of SDS micelles. On the boundary of these zones, linezolid and its impurities were solubilized by SDS micelles. The analytes, were incorporated in negatively charged micelles and migrated in reversed polarity to the detector.

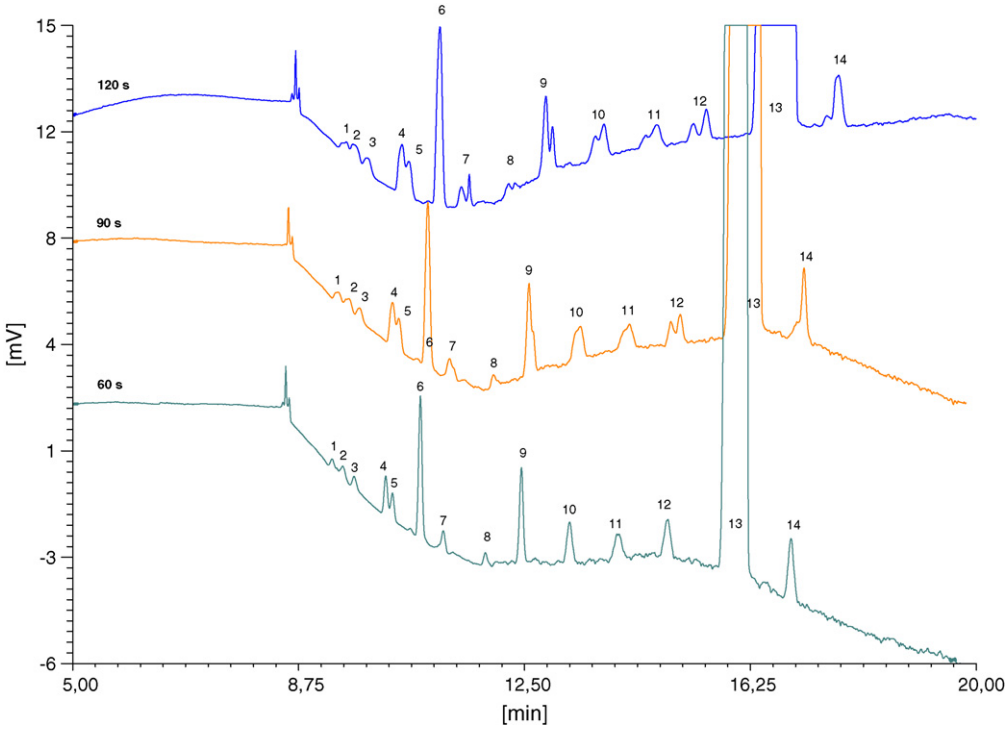

Fig. 2. Electropherograms of separation of LRS using 60 s, 90 s and 120 s sample injection, obtained under the selected conditions: 125 mM Tris/H_3PO_4 buffer, pH 2.0, 20% MeOH, 150 mM SDS (as the sweeping agent), and CE in 25 °C, uncoated capillary (50/42), reversed polarity. The identified impurities: (1)-A, (4)-G, (5)-H, (6)-I, (9)-D, (13)-linezolid.

All mentioned (below) modifications were applied with the purpoes—to improve resolution between linezolid and its achiral impurities (injected LRS).

For the first time, phosphoric acid, adjusted to pH 2.5 with addition 50% NaOH was used, but the migration time was not very encouraging (around 38 min). Therefore, the following study with TRIS, adjusted to pH 2.5 with concentrated orthophosphoric acid was performed, but without success. Next, Tris buffer was adjusted to pH 2.5 with HCl. By changing, co-ion mobilities from phosphate to chloride allowed significant shortening of migration time. Additionally, Tris/phosphate buffer with longer injections of the sample (over 60 s) result

Table 1
Characteristic of process impurities, degradation products of Linezolid, and their relative migration time, achieving according to optimized CE method

No.	Compound listed by their elution order in HPLC	No. of impurities at CE	RMT in CE	Description
1	**A**	1	0.50	Minor thermal degradation product and compound created during decomposition of D
2	B	[a]	–	Process impurity
3	C	[a]	[a]	Process impurity
4	**D**	9	0.71	Primary thermal, alkali degradation product and compound occurred in linezolid solution for infusion (2 mg/ml)
5	E	[a]	–	Process impurity
6	F	[a]	–	Process impurity
7	**G**	4	0.55	Minor thermal degradation product
8	**H**	5	0.56	Process impurity and potential acid degradation product
9	**I**	6	0.58	Primary thermal degradation product and compound occurred in linezolid solution for infusion (2 mg/ml)
10	J	[a]	–	Process impurity
11	**Linezlid, PNU-100776**	13	1.00	Biologically active substance
12	K	[a]	–	Process impurity
13	L	[a]	–	Process impurity
14	M	[a]	–	Process impurity
15	N	[a]	–	Process impurity

RMT – relative migration times in CE, according to chosen method. Acronyms in bold – compounds, which were identified by performed stress tests and separated during electrophoresis process.

[a] paeks not identified during CE stress tests.

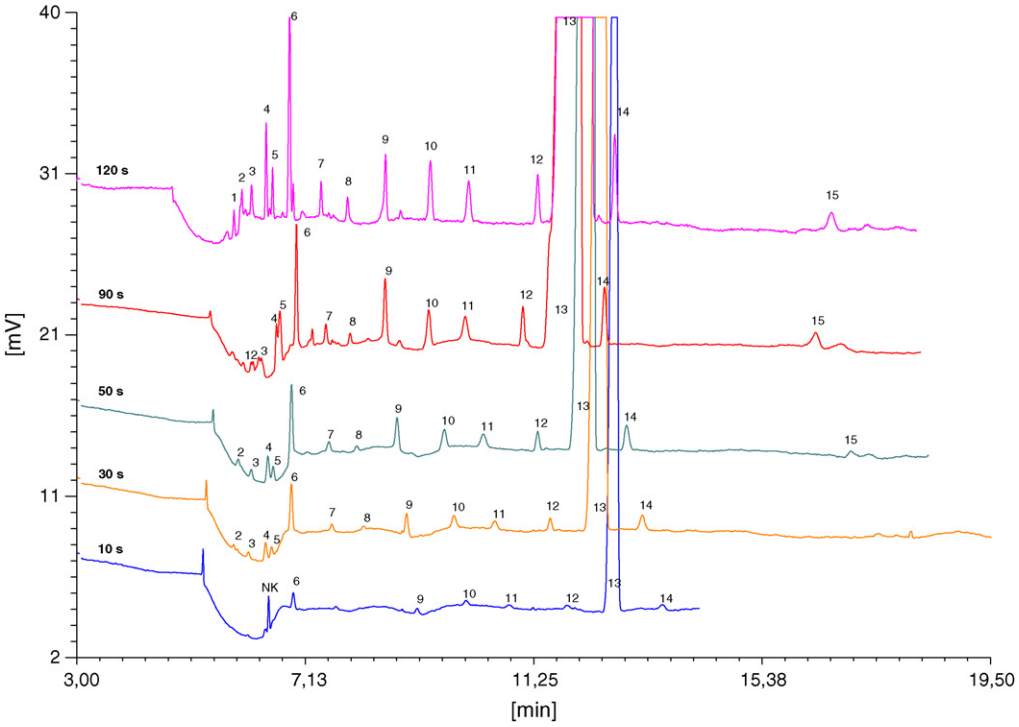

Fig. 3. Typical electropherograms of separation of LRS using 10 s, 30 s, 50 s, 90 s and 120 s sample injection, obtained under the selected conditions: 125 mM Tris/HCl buffer, pH 2.0, 20% MeOH, 150 mM SDS (as the sweeping agent), and CE in 25 °C, uncoated capillary (50/42), reversed polarity. The identified impurities: (1)-A, (4)-G, (5)-H, (6)-I, (9)-D, (13)-linezolid, NK: unknown.

in peaks distortion. Typical electropherograms were obtained under these conditions was presented in Fig. 2. Therefore optimization of sweeping conditions was performed with Tris/HCl buffer by: adding increasing concentration of Tris (tested range 50–125 mM), varied quantity of methanol or ACN (0–30%; addition of an organic solvent improves the selectivity in SDS-based MEKC), amount of surfactant (50–150 mM), temperature (15–25 °C) and value of the pH (2.0–3.0) of the buffer, while the sample (devoid of micelle) remains unchanged.

Characteristic of process impurities, degradation products of linezolid are presented in Table 1. The best results of resolution were obtained in the following conditions: 125 mM TRIS, adjusted to pH 2.0 with concentrated HCl and addition of 20% of methanol, and 150 mM SDS as a sweeping agent, CE in 25 °C,

Table 2
Linezolid Resolution Solution, Relative Migration Time, repeatability of corrected area (close to LOQ for impurities) and appropriate sweeping enhancement factor, achieved according to CE method

Peak's no. according to elution order in CE	Compound	RMT in CE	Repeatability of corrected area (R.S.D%)	SFE (peak height)	SFE (peak area)
1	A	0.50	NC	[a]	[a]
2	•	0.51	7.27	[a]	[a]
3	•	0.53	7.45	[a]	[a]
4	G	0.55	3.70	[a]	[a]
5	H	0.56	5.78	[a]	[a]
6	I	0.58	4.10	5.3	9.9
7	•	0.62	1.42	[a]	[a]
8	•	0.66	2.35	[a]	[a]
9	D	0.71	3.57	12.1	8.9
10	•	0.78	1.82	12.5	10.0
11	•	0.83	0.68	13.3	10.0
12	•	0.93	2.43	12.6	8.6
13	Linezlid, PNU-100776	1.00	0.49	3.6	9.52
14	•	1.03	1.94	17.6	11.4
15	•	1.34	8.57	[a]	[a]

SFE – sweeping enhancement factor – calculated as a ratio of peak height/corrected area of the peak obtained when sweeping was performed (120 s) to peak height/corrected area obtained when sweeping was performed for 10 s. (•) peak no identified during CE stress tests. NC: not calculated.

[a] lack of peaks under 10 s injection.

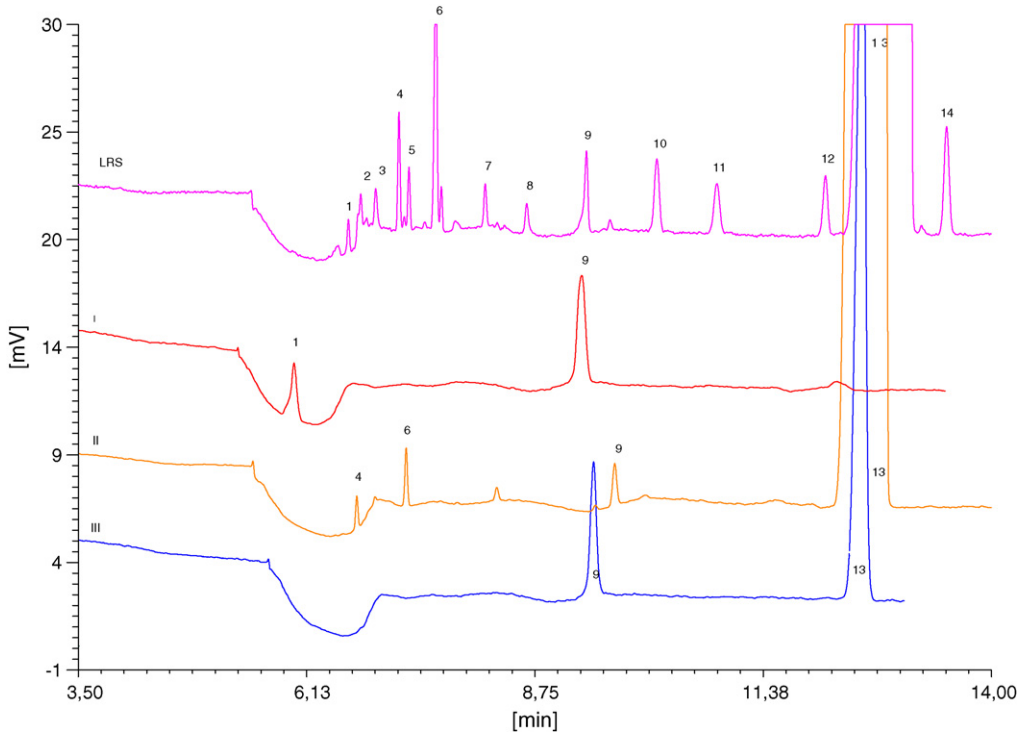

Fig. 4. Electropherograms demonstrating investigations of degradation products after stress tests: base and heat treatment of linezolid, obtained under the selected conditions: 125 mM Tris/HCl buffer, pH 2.0, 20% MeOH, 150 mM SDS (as the sweeping agent), and CE in 25 °C, uncoated capillary (50/42), reversed polarity. The identified impurities: (1)-A, (4)-G, (5)-H, (6)-I, (9)-D, (13)-linezolid. Typical electropherogram of unstressed LRS (top) and base + heat (I), heat (II), base (III) sample.

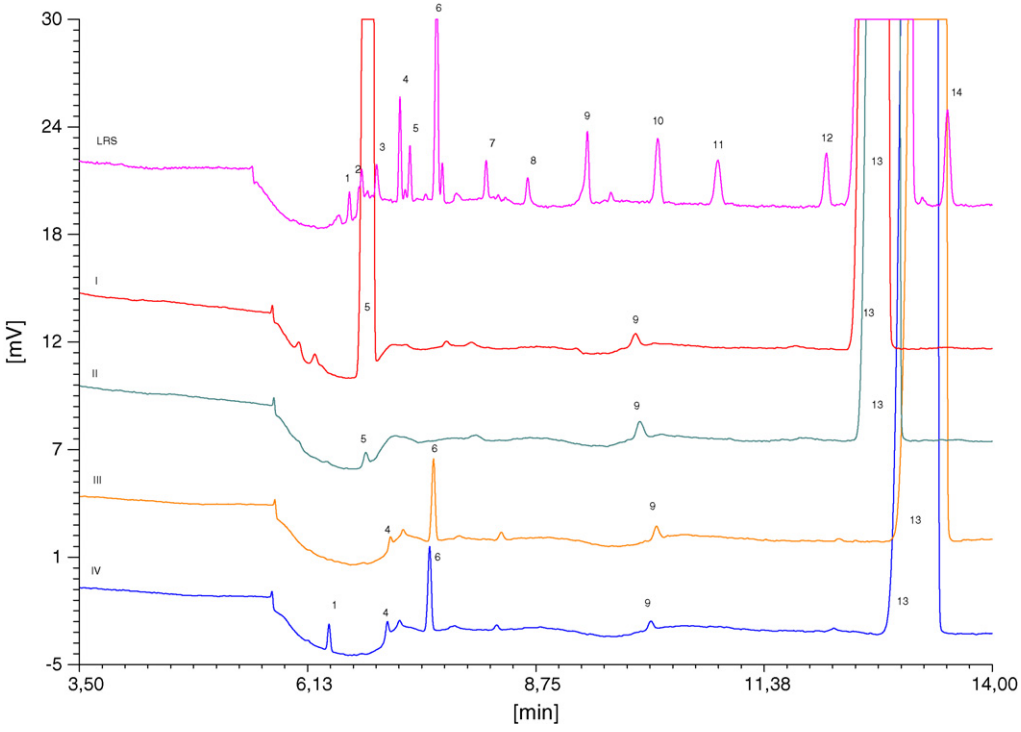

Fig. 5. Electropherograms demonstrating investigations of degradation products after stress tests: acid, base, heat treatment of linezolid. Experimental conditions as in Fig. 4. The identified impurities: (1)-A, (4)-G, (5)-H, (6)-I, (9)-D, (13)-linezolid. Typical electropherogram of unstressed LRS (top), acid + heat (I) acid (II), linezolid, solution for infusion (III) and linezolid solution for infusion, heated (IV) sample.

uncoated capillary (50/42), reversed polarity (15 kV), injection 120 s (Fig. 3). Sweeping measurements were performed after 10, 20, 30, 40, 50, 70, 90 and 120 s sample injection time. Some of the results are depicted in Fig. 3. Longer injections caused an increase of peak area and at the same time, a decrease of migration time. It is clear from the electropherograms that all impurities are well resolved from linezolid as well as from each other. We did not observed deterioration of separation efficiency with longer injection time (120 s injection), all signals still were well separated.

Satisfactory separation was possible after 19 min of electrophoresis. Peaks distortion as a result of longer sample injections was not observed. The peaks were narrow, with high signals, what is known as an advantage of low EOF techniques [33].

Sweeping enhancement factors (SEF) for all by-process and degradation products occurring in resolution solution of linezolid, calculated as a ratio of peak height and corrected area of the peak obtained when sweeping was performed (120 s) to peak height and corrected area obtained when sweeping was performed for 10 s; are presented in Table 2.

3.2. Stress tests

Due to the fact, that only a mixture of linezolid and its achiral impurities (LRS) was available, stress tests have been performed to identify degradation products for linezolid solution at concentration 0.32 mg/ml. Investigations of degradation products due to stress tests: acid (1 M HCL; 1 M HCL + 95 °C, incubation time around 30 min), base (0.1 M NaOH, 1 M NaOH + 95 °C, incubation time around 30 min) and heat treatment (60, 70 and 95 °C) have been performed and appropriate electropherograms are presented in Figs. 4 and 5. According to impurities characteristic included in manufacturer documentation as well as simultaneously performed HPLC assay (according to producer validated method) and CE analysis, identification of particular degradation products of linezolid (A, D, G, H, I) was possible. During acid degradation linezolid decomposed into H, which was a potential acid degradation product. During thermal and alkali degradation—D, forms initially in 0.1 M NaOH but then decomposes to A. Under thermal stress tests, two major impurities I and D as well as two minor impurities, A and G of linezolid were formed. Samples of linezolid solution (0.32 mg/ml) were treated by high temperature: 60, 70 and 95 °C. At lower temperatures (60 °C), peak area of I was greater than D. At 70 °C, the rates of I and D formations were nearly equivalent. At 95 °C the rate of appearance of D exceeded that of I. It is obvious, that formation of thermal degradation products were temperature dependent. The results of our experiments (Figs. 4 and 5) agreed, with manufacturer's impurities characteristic and HPLC analysis. The identified impurities were listed in Table 2, by their elution order, according to CE method.

Unfortunately in this way it was impossible to confirm by-products from the synthesis route: B, C, E, F, J, K-N.

Therefore to ensure that no compounds coelute with linezolid, a peak homogeneity assessment was performed by Agilent HP3D system, equipped with photodiode array detector.

Table 3
Comparison of CE method (day 1, day 2) for determination of linezolid in preparation: linezolid solution for infusion (2 mg/ml)

	CE assay (day 1)	CE assay (day 2)
Results	100.94	103.21
	100.23	104.56
	102.48	102.23
	102.88	102.83
	103.63	104.21
Mean (%)	102.03	103.41
S.D	1.407	0.966
R.S.D. (%)	1.379	0.934
Student's t-value for $P = 0.05$, $n = 10$, t tabulated = 2.306	1.746	

3.3. Peak purity analysis

DAD was used to determine peak purity by determining spectral homogeneity across the peak of linezolid. In all analyses the wavelength region used, was 190–390 nm. During this experiment we compared peak of reference spectrum of linezolid with linezolid apex spectrum of the peak achieved during electrophoresis process of impurities mixtures (Linezolid Resolution Solution). Result-peak of linezolid achieved during electrophoresis (optimized method) was pure, what means that impurities were well separated from the main peak (linezolid).

High probability of peak purity was achieved, taking into account, that determining peak purity by peaks spectra never proves chemical purity of the peak in cases: when resolution between the analyte and the impurity is $R < 0.7$, when, very similar spectra for structurally related compounds are observed and in case, when the impurity presented is below the limit of detection.

3.4. Validation

Optimized method was validated in terms of the detection limit (LOD), the quantification limit (LOQ), linearity, precision and accuracy, according to European Medicinal Agency CPMP/ICH/381/95 directive.

Linezolid samples stored in the solid state (tablets and oral suspension) have shown no evidence of significant linezolid degradation products. Degradation occurs only in solution (linezolid solution for infusion—2 mg/ml): I and D, therefore to

Table 4
Comparison of CE and HPLC methods for determination of linezolid in preparation: linezolid solution for infusion (2 mg/ml)

	CE assay	HPLC[a] assay
Mean (%)	102.03	101.01
No. of samples	5	5
S.D.	1.407	1.06
R.S.D.(%)	1.379	1.049
Student's t-value for $P = 0.05$, $n = 10$, t tabulated = 2.306	1.297	

[a] The HPLC assay was performed according to manufacturer method (binary gradient, analysis time around 45 min).

Table 5
Comparison of CE and HPLC methods for determination of related compound in preparation: linezolid solution for infusion (2 mg/ml)

Impurities	CE			HPLC		
	I	D	Sum	I[a]	D[b]	Sum
Mean (%, $n = 10$ CE, $n = 9$ HPLC)	0.57	0.11	0.91	0.52	0.096	0.80
S.D	5.52×10^{-3}	0.0195	0.051	0.028	9.89×10^{-3}	0.036
R.S.D. (%)	0.97	17.17	5.61	5.46	10.28	4.51

[a] For the calculation of contents impurity I peak area was divided by correction factor 0.7 (according to manufacturer method).
[b] For the calculation of contents impurity D peak area was divided by correction factor 1.1 (according to manufacturer method).

validate the optimized method, linezolid solution for infusion (2 mg/ml) was selected.

3.4.1. Sensitivity

LOD and LOQ defined as signal to noise ratio of 3:1 and 10:1, respectively, were calculated according to the formulas: (cxh/2H)3 and (cxh/2H)10, where c is a concentration (mg/ml), *h* is height of noise signal and H is an analyte peak height. LOD for linezolid was calculated at the level of 0.05 μg/ml, LOQ was 0.14 μg/ml.

3.4.2. Linearity

In order to show that the electrophoretic system produces a linear response to linezolid over the expected range of use, a linearity study was performed. The preparation – linezolid solution for infusion (2 mg/ml) was used to prepare dilutions in the range from 0.0005 to 0.32 mg/ml. A total of five discrete concentrations were prepared and analyzed. The detector response was examined by linear regression ($y = 1 \times 10^6 X + 9425.5$) – correlation coefficient of 0.9988 was calculated. Each sample concentration was injected twice.

3.4.3. Precision

Instrumental precision was calculated from three consecutive standard injections of LRS, which is a mixture of impurities with linezolid (concentration for impurities were close to LOQ value, while linezolid was at concentration of 0.32 mg/ml), and R.S.D. of area counts for particular impurities were presented in Table 2. Instrumental precision for linezolid at concentration close to LOQ value was calculated from five consecutive injections, and R.S.D. of area counts for linezolid was 1.65%. Independent assays performed by two analysts on different days, confirmed good repeatability and intermediate precision (Table 3). Results of linezolid content did not differ significantly. The Student's *t*-values determined by the *t*-test, were lower than theoretical (tabular) value.

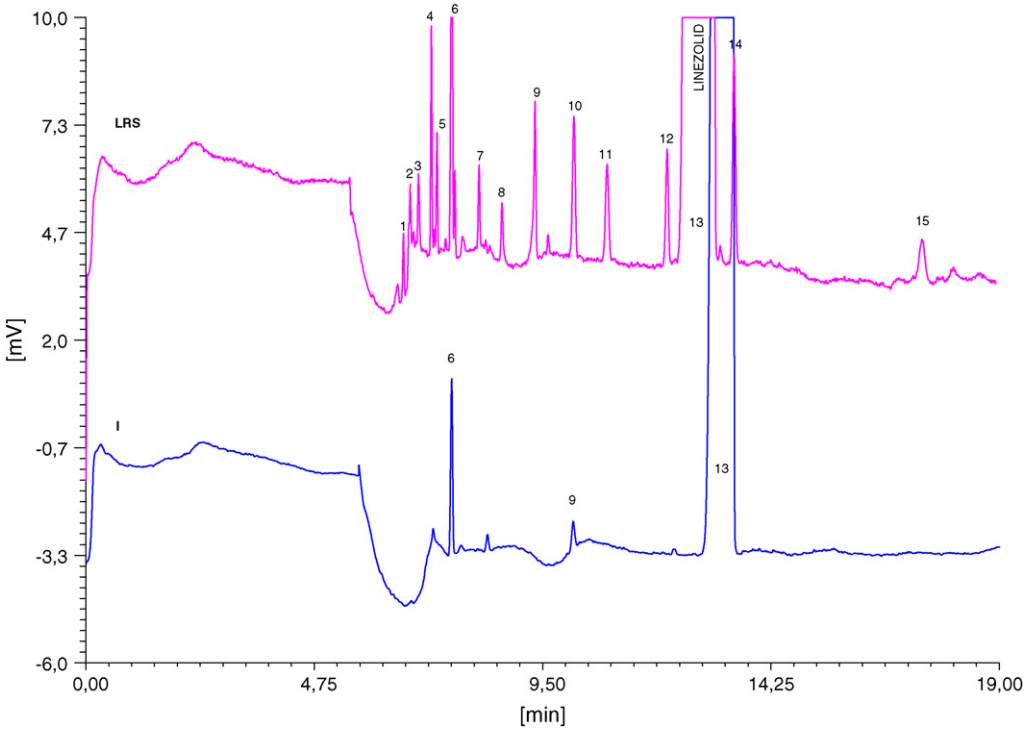

Fig. 6. Electropherograms demonstrating separation of LRS (top) and typical electropherogram of preparation sample – linezolid solution for infusion. Experimental conditions as in Fig. 4. The identified impurities: (1)-A, (4)-G, (5)-H, (6)-I, (9)-D, (13)-linezolid.

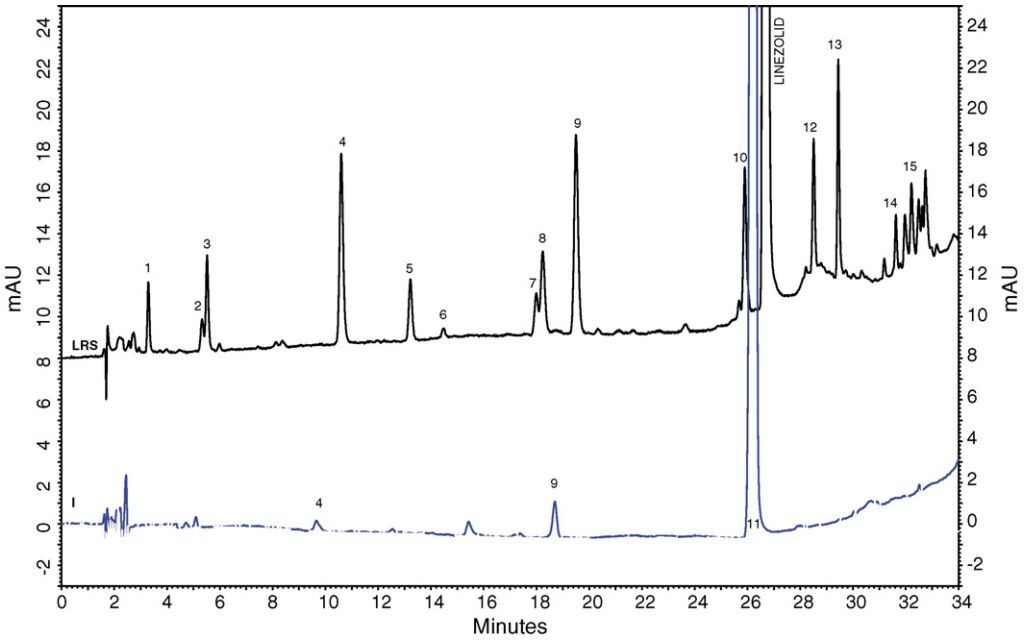

Fig. 7. HPLC chromatogram demonstrating separation of LRS (top) and typical chromatogram of sample linezolid solution for infusion obtained according to producer validated method (binary gradient, analysis time around 45 min). The identified impurities: (1)-A, (2)-B, (3)-C, (4)-D, (5)-E, (6)-F, (7)-G, (8)-H, (9)-I, (10)-J, (11)-linezolid, (12)-K, (13)-L, (14)-M, (15)-N.

3.4.4. Accuracy

During further experiments, accuracy reported as the comparable assays by CZE and HPLC methods, were performed.

The results are presented in Tables 4 and 5. Independent assays performed by CE and HPLC confirmed good accuracy. Results of linezolid content did not differ significantly. The Student's *t*-values determined by the *t*-test, were lower than theoretical (tabular) value, indicating that there was no statistically significant difference between the two methods.

The amount of impurities occurred during electrophoresis process in the test solution were calculated as ratio of impurity peak in the electrophoregram obtained with test solution to sum of all areas of all peaks in the electropherogram obtained with test solution and then multiplied by 100%.

The results obtained from both methods confirmed the presence of two impurities in drug formulation - linezolid solution for infusion (2 mg/ml): I and D. Figs. 6 and 7 illustrate appropriate electropherogram and chromatogram, respectively. Under applied experimental conditions, UV–vis absorbance detection in CZE (with sweeping) had a good sensitivity, when compared to HPLC method sensitivity (LOD – 0.02 μg/ml; LOQ – 0.08 μg/ml).

4. Conclusions

The application of a sweeping preconcentration enabled fast, simple and effective separation of linezolid from its achiral impurities by CE using UV absorption detection method. Satisfactory separation was possible after less than 19 min of electrophoresis. Peaks distortion as a result of longer sample injections was not observed—the peaks were narrow, with high signals.

Independent assays performed by CE and HPLC confirmed good accuracy. Results of linezolid content did not differ significantly. The Student's *t*-values determined by the *t*-test, were lower than theoretical (tabular) value, indicating that there was no statistically significant difference between the two methods.

Unfortunately, separated, known impurities were unavailable from Pfizer thus full validation procedure for impurities: linearity and sensitivity was not performed. However, confirmation of selectivity of the elaborated method of peak purity measurements was achieved.

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