Acta Poloniae Pharmaceutica – Drug Research, Vol. 70 No. 2 pp. 171–180, 2013 ISSN 0001-6837
Polish Pharmaceutical Society

REVIEW

CAPILLARY ELECTROPHORESIS APPLICATION IN THE ANALYSIS OF THE ANTI-CANCER DRUGS IMPURITIES

MARTA ZALEWSKA[1]*, KAROL WILK[2] and HALINA MILNEROWICZ[1,3]

[1]Department of Biomedical and Environmental Analysis, [2]Students Scientific Association, Department of Biomedical and Environmental Analysis, Faculty of Pharmacy, Wroclaw Medical University, Grunwaldzka 2, 50-355 Wrocław, Poland
[3]Department of Basics of Physiotherapy, University School of Physical Education, Wrocław, Poland

Abstract: Due to the low therapeutic index of anti-cancer drugs, they should be closely monitored for evidence of potential contamination that may be of high toxicity and not to have the desired therapeutic effect. Therefore, analytical methods to detect drugs related substances at low concentrations are necessary. Capillary electrophoresis allows for fast and clear separation of drug derivatives. A multitude of submethods make selection of suitable environment for various types of chemicals possible. Publications concerning separation of drugs such as cisplatin, carboplatin, lobaplatin, methotrexate, tamoxifen, paclitaxel from their derivatives, which are their potential contaminations, show that capillary electrophoresis provides the appropriate tools to analyze the impurities of these anti-cancer drugs and is able to partially displace such technique as thin layer chromatography and high performance liquid chromatography, which still play a major role in this field.

Keywords: capillary electrophoresis, anti-cancer drugs

Analysis of purity is one of the steps that each drug must pass before it is registered. Impurities can be both substances produced during the manufacturing process and products of degradation of the active ingredients. Safety of the therapy is closely related to the quality of medication. Undesired components may potentiate the toxicity of the drug, having its own toxicity and lead to the lost of therapeutic properties of the substance. This is the reason why the analysis of the purity has become one of the most important steps in ensuring the quality of drugs (1). The determination of impurities in drugs is a challenge because they can be quite complex. Substances of highly similar structure to the main drug are usually involved, what necessitates a highly selective separation process. Within impurity profiling, it is important to reach as many separated peaks as possible, which is facilitated by high efficiencies.

Particular attention should be noted to anticancer preparations, as they contain substances that exhibit a high toxicity, and their degradation products can have powerful pharmacological properties often leading to an increase in the toxicity. Derivatives impurities are known to have different activities and may possess genotoxic activity (2, 3).

There are many analytical techniques used in pharmaceutical laboratories in the analysis of the drugs impurities. One of them is capillary electrophoresis (CE). This method became popular in the early eighties, after the Jorgenson and Lukacs demonstration that the effectiveness of the electrophoresis may be increased if it will be done in quartz capillaries with a diameter of ~75 μm (4). These experiments resulted in the rapid development of CE techniques, which make it a very attractive alternative to other chromatographic techniques. CE is a highly sensitive and effective analytical technique high resolving, that can be applied for the separation of a large variety of substances, from ions and small molecules to high molecular size macromolecules. The combination of both electrophoretic and chromatographic mechanisms of migration, enabled the development of different separation CE modes. The variety of CE modes and selectivities that can be applied, improve separation capability. Depending on charge density, size, hydrophobicity and chirality, CE can be applied to different category of chemicals.

* Corresponding author: e-mail: zalewska.m@gmail.com; phone: +48 71 7840173; fax: +48 71 7840172.

This review illustrates possible applications of CE in anti-cancer drugs impurity profiling. Due to the large peak capacity of the technique, it is well suited to separate the main drug compound from its possible impurities that often have very related chemical structure.

Capillary electrophoresis methods

A multitude of CE applications is a consequence of the presence of many varieties that differ in terms of particle separation process: capillary zone electrophoresis, capillary gel electrophoresis, micellar electrokinetic capillary chromatography, microemulsion electrokinetic chromatography, capillary electrochromatography, capillary isoelectric focusing, capillary isotachophoresis and non-aqueus capillary electrophoresis.

Electroosmosis plays an important role in the CE. Silanol groups are dissociating, forming negative charge in the inner surface of capillary, which attracts positive charges from the buffer filling the capillary, forming an electric double layer. The solution, which borders on the surface of the capillary, contains an excess of positive ions. When a voltage is applied, electric field is created, what causes the movement of a layer of ions towards the cathode, resulting in the filling the capillary with solution. Electroosmotic flow is strong enough to cause movement of cations and also neutral molecules toward the cathode (6, 7). The three main separation mechanisms employed are low pH (in the analysis of basic drugs), high pH (in the analysis of acidic drugs) and micellar electrokinetic capillary chromatography. Also, various additional separation mechanisms have been employed to expand separation options (8). Diverse additives, as methanol or acetonitrile (ACN), can be added to the main buffer to improve selectivity by altering viscosity and polarity of the buffer. In the consequence, electroosmotic flow and the electrophoretic mobility of the analyzed substance can be affected. Addition of cyclodextrins (CDs) can improve resolution (9).

CE has been employed to determine related impurities of the wide range of drugs. Capillary zone electrophoresis (CZE) is the simplest form of CE. Electrophoretic mobility of particles depends on the difference of their charge to mass ratio. Capillary is filled with a buffer, and the applied voltage is uniform throughout its entire length (5). CZE is used for separation of low molecular weight proteins, peptides, amino acids, catecholoamines (7, 10). CZE is also used in the analysis of drugs purity such as tetracycline, ciprofloxacin, erythromycin, ranitidine, buspirone, naphazoline, ephedrine, morphine (9).

Capillary gel electrophoresis (CGE) is a method in which the capillary is filled with an agarose or polyacrylamide gel. The separation is a consequence of the difference in the size and shape of charged particles, which migrate at different rates (11). Sample introduction can be done only by electrokinetic injection, not by hydrodynamic methods because the capillary is plugged with the gel. CGE is used for the separation of compounds with similar charge-to-mass ratios, such as SDS-proteins complexes and nucleic acids (9).

Micellar electrokinetic capillary chromatography (MEKC) was introduced for the separation of neutral molecules. In this method, capillary is filled with a buffer containing surfactant at concentration above the critical micellar concentration, which allows the formation of micelles, and creates a pseudostationary phase enabling high-efficiency chromatographic separations. During the separation, nonpolar molecules penetrate into the interior of micelles, and polar molecules remain in the buffer (11). Drug-related impurities tend to have similar charge to mass ratios to each other, and MEKC is more appropriate, because drugs impurities can be separated with improved selectivity by a combination of charge to mass ratio, hydrophobicity, and charge interactions at the surface of the micelles. This method was applied for determination of homocysteine, bile acids (7, 10) and the analysis of the purity of drugs such as diltiazem, mebeverine, didanosine, amoxicillin, cefradine, erythromycin, cisplatin, carboplatin, paclitaxel, methotrexate (9).

Microemulsion electrokinetic chromatography (MEEKC) was introduced as an alternative to MEKC. It allows for the separation of both hydrophobic and hydrophilic particles. As a supplement to the buffer, the anionic surfactant is used (for example sodium lauryl sulfate), which is able to form the microemulsion and co-surfactant such as short-chain alcohol, which stabilize the emulsion (11). Microemulsions contain nanometer-sized oil droplets suspended in an aqueous buffer and the surface tension between the oil and water components is reduced by covering the oil droplet with an anionic surfactant. This method is used in the analysis of drugs purity such as ranitidine, tolbutamide, cefuroxime, naproxen (9).

Capillary electrochromatography (CEC) is a hybrid technique that combines the features of liquid chromatography (LC) and capillary electrophoresis. Substances are separated according to their partitioning between solid and mobile phase. In the case of substances with charge, their mobility in an elec-

tric field is also used (6, 7). CEC is well suited for drugs impurity profiling because of its advantages of high selectivity of LC and high efficiency of CE. This method is utilized in the analysis of purity of fluticasone, cefuroxime, tetracycline (9).

Capillary isoelectric focusing (CIEF) is a technique that uses the differences in particles separation caused by the values of the isoelectric point. In the capillary, the pH gradient is created using zwitterionic molecules called carrier ampholites - at the anode pH is low, and at the cathode is high. Substances migrate up to the pH corresponding to their isoelectric point. This technique allows the separation of amphoteric substances. Its main drawback is the inability to separate substances of similar isoelectric point (12).

Capillary isotachophoresis (CITP) is a technique that uses two different electrolytes: leading - containing ions of greater mobility than sample ions, ending - with lower ion mobility. When a voltage is applied, ions migrate between the leading and ending electrolyte in order of their electrophoretic mobility (11). This method has been used in the analysis of human hemoglobins.

Non-aqueous capillary electrophoresis (NACE) is used for the separation of hydrophobic compounds, which are difficult to be analyzed in aqueous media due to their low solubility. As basic solutions, organic solvents: methanol, ACN, ethanol, formamide, dimethyl formamide are used. Using of organic solvents in the buffer results in reduced Joule heating and low current, what allows the use of high electric field strength (9). When NACE method is used, evaporation of the electrophoresis medium has to be taken into account. Organic solvents that exhibit strong UV absorbance require the use of indirect UV or electrochemical detection, and MS detection (9). NACE can be used in the analysis of drugs such as bromazepam, nicotine, fluoxetine, tamoxifen (13).

Capillary electrophoresis application in the analysis of the anti-cancer drugs impurities

To ensure the effectiveness of drug and minimize the side effects, it is necessary to perform its quality control. Regulatory guidelines issued by the International Conference on Harmonisation demand that impurities in newly synthesized compounds are investigated from a certain limit upwards (14, 15). In accordance with these standards, when the daily dose is equal to or less than 2 g/day, any contamination present in an amount equal to or greater than 0.05% should be reported. When the amount is 0.10%, impurity must be identified, and at 0.15% must be classified toxicologically. For daily doses exceeding 2 g/day, these criteria are reduced sequentially to 0.03%, 0.05% and 0.05% (2, 14, 15). Techniques used in the analysis of purity of anti-cancer drugs are: high performance liquid chromatography (HPLC), thin layer chromatography (TLC) and gas chromatography (GC). Polish Pharmacopoeia VIII does not currently provide application of CE in the analysis of anti-cancer drugs impurities (16). This situation may change with the increasing number of publications with the use of CE on the distribution of drugs and substances related to them, which could pose a potential contamination.

Analysis of the purity of platinum complexes

Chemotherapy with platinum metallodrugs is a well-established treatment option for patients suffering from different types of cancers. Cisplatin, carboplatin, and oxaliplatin are the most important examples of the compound class. Their mode of action was found to be related to the ability to bind to DNA and modify its structure, what leads finally to induction of apoptosis/necrosis (17).

Cisplatin ((SP-4-2)-diamminedichloridoplatinum) (Fig. 1a) is known to form DNA adducts and, thus, damage of DNA is implicated in its anti-tumor activity. Cisplatin and to a greater extent its hydrol-

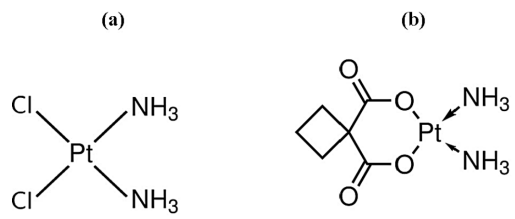

Figure 1. Structural formula of **a**) cisplatin ((SP-4-2)-diamminedichloridoplatinum) and **b**) carboplatin [cis-diammine(cyclobutane-1,1-dicarboxylate-O,O') platinum(II)] (19, 22)

ysis product monohydrated cisplatin are responsible for side-effects like neuro- and nephrotoxicity. The methods used for determination of cisplatin are: HPLC in combination with different types of on- and off-line detectors including UV detection, flame atomic absorption spectroscopy, inductively coupled plasma atomic emission spectrometry, radioactivity detection and electrochemical detection (18). In order to reduce side effects and also increase the water solubility and stability, new drugs are synthesized, that differ from cisplatin in the side chains. These include carboplatin and lobaplatin (19). For both, cisplatin and carboplatin, it is known that the *cis*-diammineplatinum(II) species have cytotoxic activity while the *trans*-diammineplatinum(II) species do not. The difference in anti-tumor activity between the two isomers is attributed to the inability of the *trans* isomer to form 1,2-GpG intrastrand crosslinks due to the 180^{o} angle between its two semi-labile chloride or carboxylate ligands (20).

The main impurities that may be present in the formulations of cisplatin, carboplatin and lobaplatin are the products of their hydrolysis. Cisplatin first hydrolyzes to monoaquacisplatin, and then to diaquacisplatin. Carboplatin (Fig. 1b) is hydrolyzed in turn to diaquacisplatin and the anion of 1,1-cyclobutane dicarboxylate. Polish Pharmacopoeia VIII as a technique used for analysis the purity of cisplatin describes the TLC (16), and to analyze the purity of carboplatin - LC (16).

Wenclawiak and Wollman describe the use of MEKC technique for separation of cisplatin, carboplatin and lobaplatin from their hydrolysis products (19). As a basic solution 25 mM $Na_2B_4O_7$ – 50 mM NaH_2PO_4; pH 7.0 was used. Melted silica capillary with a length of 50 cm to the detector was used and the applied voltage was 15 kV. Measurements were performed at a wavelength 210 nm (19). Sodium dodecyl sulfate (SDS) concentration affects the efficiency of separation and the best SDS concentration for cisplatin separation was 100 mM, and for loboplatin and carboplatin was 80 mM. Cisplatin and carboplatin do not have very lipophilic structure, and therefore weakly interact with the micelles. An important fact is that the hydrolysis products of cisplatin despite having a positive charge, exhibit an electrophoretic mobility towards the anode, because of their interaction with the negatively charged surface of the micelle. However, analyzed particles migrate towards the cathode due to the electroosmotic flow dominance on the electrophoretic current. In the case of lobaplatin, efficiency of peaks separation increases with increasing SDS concentration and decreasing voltage (19). The proposed method allows the cisplatin separation from its hydrolysis products, what allows the study of cisplatin preparations for evidence of its impurities. This method also allows the separation of diastereomers lobaplatin and carboplatin from their hydrolysis products. To carry out these separations, it was necessary to add NaCl to the solution (4 mM NaCl in the case of lobaplatin and 10 mM NaCl for carboplatin).

The presented method offers new possibilities in the study of complex platinum compounds such as: the study of their stability, their hydrolysis kinetics and reactivity. The main advantage of this method is a separation of neutral particles from

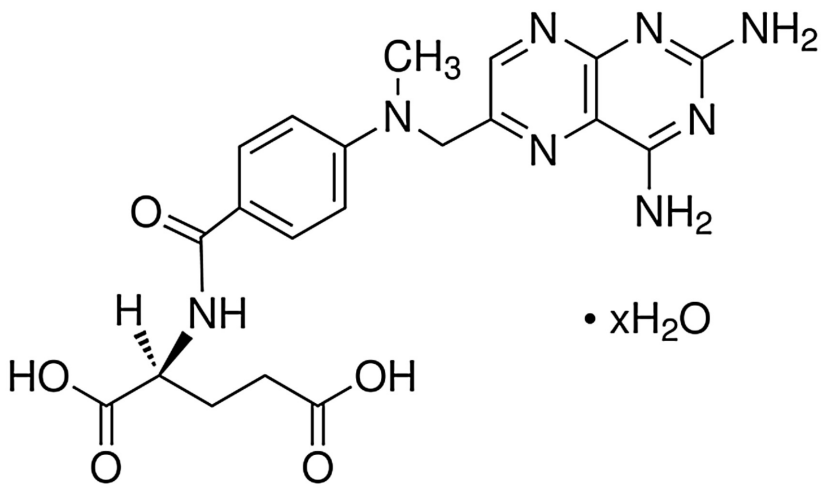

Figure 2. Structural formula of methotrexate (16)

complexes with charge in a single measurement, while the using for example HPLC requires several measurements using different techniques (19).

Analysis of methotrexate purity

Methotrexate (MTX, (2S)-2-[(4-{[(2,4-diaminopteridin-6-yl)methyl](methyl)amino}(benzoyl)amino]pentanedioic acid) (Fig. 2) is a drug routinely used in anti-cancer therapy as an anti-metabolite of folic acid. Dextrorotatory enantiomer of MTX differs from the levorotatory form in the pharmacokinetic properties. Its bioavailability is only 2.5% of the bioavailability of L-MTX, which is why it is recognized as a chiral impurity (21). The optical purity of MTX was assessed by means of different techniques; the use of amperometric biosensors and HPLC methods, showed to be useful in pharmaceutical applications. None of the reported methods was applied to the simultaneous quantification of the chiral and achiral impurities of MTX. Others potential contaminations of MTX are: 2,4-diamino-6-(hydroxymethyl)pteridine, aminopterin, 4-[N-(2-amino-4-hydroxy-6-pteridinylmethyl)-N-methylamino] benzoic acid, 4 [N-(2,4-diamino-6-pteridinylmetyl)-N-methylamino]benzoic acid (Fig. 3).

Polish Pharmacopoeia VIII as a technique for the determination of MTX impurities indicates LC (16). Gotti et al. described the MTX separation from its impurities by the MEKC technique, which may be the competitive technology to that mentioned in pharmacopoeia (21).

The accuracy of separation is also influenced by SDS, cyclodextrins (CDs), and methanol concentration, and therefore, to optimize the separation

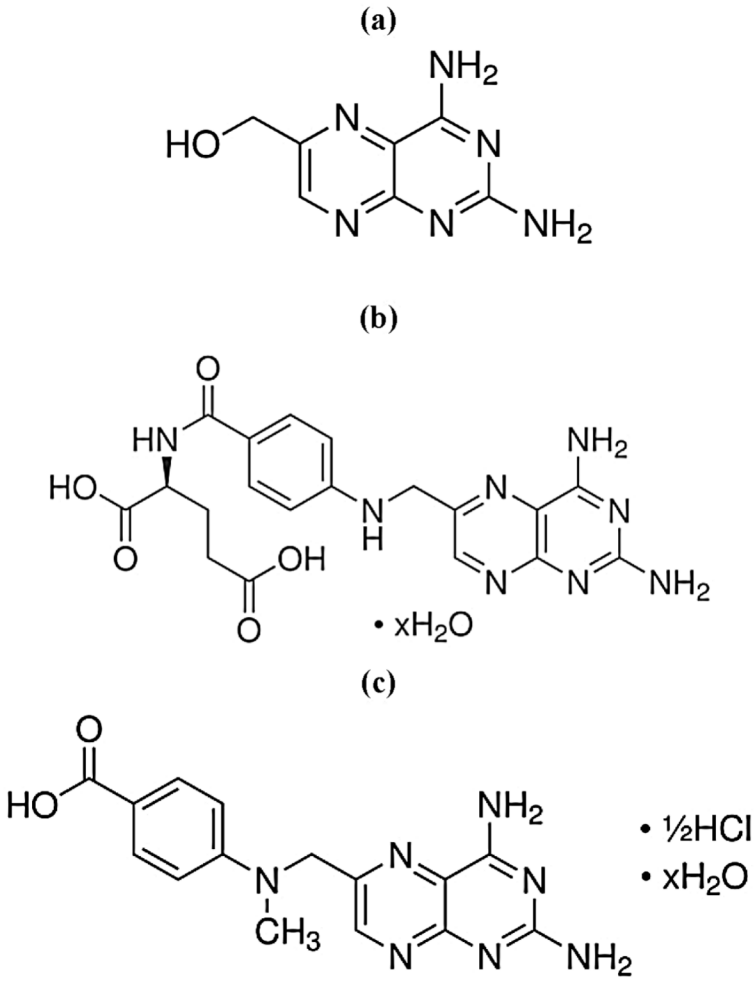

Figure 3. Structural formulas of methotrexate potential impurities: (**a**) 2,4-diamino-6-(hydroxymethyl)pteridine, (**b**) aminopterin hydrate, (**c**) 4-[*N*-(2,4-diamino-6-pteridinylmethyl)-*N*-methylamino] benzoic acid (21)

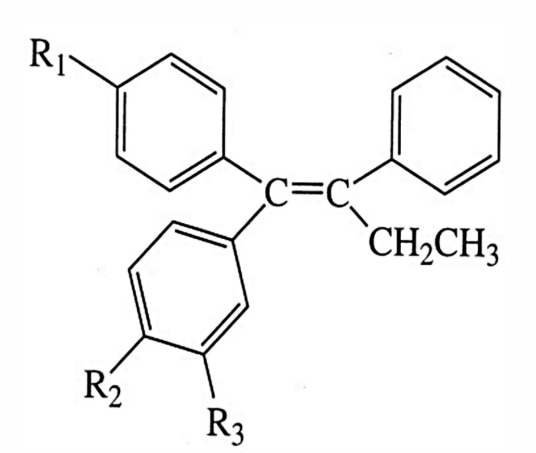

Figure 4. Structural formula of tamoxifen. Substituents tamoxifen degradation products : monohydroxytamoxifen (R_1 - $(CH_3)_2N(CH_2)_2O$-; R_2 - HO-; R_3 - H-); dihydroxytamoxifen (R_1 - $(CH_3)_2N(CH_2)_2O$-; R_2 - HO-; R_3 - HO-); decomposition product A (R_1 - HO-; R_2 - H-; R_3 - H-); decomposition product B (R_1 - $(CH_3)_2N(CH_2)_2O$-; R_2 - H-; R_3 - H-) (22, 23)

conditions, several measurements at different concentrations of the above-mentioned substances have been performed. An increase of the SDS concentration from 50 to 100 mM allows for better separation of 2,4 diamino-6-(hydroxymethyl)pteridine from the peak resulting from electroosmotic flow. It also allows the initial separation of the aminopterin from the racemic mixture of MTX, therefore, for further studies, SDS concentration equal to 100 mM in the basic solution was used (21). As a substance intended to provide a chiral separation of the MTX, racemic mixture of CDs was used. The main solution contained two "quasi stationary" phases with a different mobility and different ability to interact with the tested substance: separation of substances soluble in micelles and the inclusion complexation of CDs (21). The use of β-CD resulted in higher enantioselectivity, than the use of hydroxypropyl-β-CD (HP-β-CD). Increased concentrations of β-CD from 30 to 45 mM caused an increase in migration time of all components of the mixture and decreased the electroosmotic flow strength. This is indicated by an increased migration time of electroosmotic flow peak and increased resolution allowing for aminopterin and 4-[N-(2-amino-4-hydroxy-6-pteridinylmethyl-N-methylamino]benzoic acid separation, what was not possible at lower concentrations of β-CD.

Resolution of received signal during separation was also modified by the addition of organic solvent to the main solution. Among tested solvents, it was methanol which gave the best results. The use of higher concentrations of methanol resulted in an increased resolution and lengthening the time of separation (21). Experimentally was established that the optimal conditions for separation of MTX impurities are: 100 mM SDS, 45 mM β-CD in 50 mM borate buffer of pH 9.3 containing 25% methanol. In such conditions separation of L-MTX at concentration 2.5 mg/ml was conducted, to which 0.2% of the potential contamination was added, and then analyses of the purity of MTX: solution for injection and tablets were performed (21). As resulted from the obtained electropherograms, solution for injection contained 1.80% impurity of dextrorotatory enantiomer of MTX. Interestingly, the tablets were less contaminated by D-MTX (1.11%), but contained trace amounts (0.008%) of aminopterin (21).

Analysis of purity of tamoxifen

Tamoxifen (Z)-2-[4-(1,2-diphenylbut-1-enyl) phenoxy]-N,N-dimethylethanamine is a drug widely used in the treatment of breast cancer. Tamoxifen and its analogs are conventionally analyzed by HPLC. Polish Pharmacopoeia VIII as a technique for the determination of impurities, which are tamoxifen related substances, also recommends HPLC (16). This technique is sensitive but time-consuming and uses large volumes of solvents.

Xing-Fang Li et al. proposed another method of tamoxifen separation from the products of its decomposition: monohydroxytamoxifen, dihydroxytamoxifen, [trans-1-hydroxy-1,2-diphenylbut-1-ene (decomposition product A) and trans-1-(4-β-methyloaminoetoxyphenyl)-1,2-diphenylbut-1-ene (decomposition product B) (Fig. 4) (22, 23). Proposed technique is NACE with both thermooptical absorbance detection and electrospray ionization mass spectrometry (ESI-MS) as detection methods. This technique allows the separation without the use of surfactants, which may cause slight contamination in the vacuum chamber during the electrospray ionization (23). Analyses were performed using 55 cm capillary length. As a basic solution, 20 mM ammonium acetate in a mixture of ACN and methanol in a volume ratio of 3:7, v/v was used. This method allowed the separation of tamoxifen from the products of its degradation. Individual peaks were identified using ESI-MS. By the use of NACE without surfactants, it was possible to combine CE with ESI-MS, which resulted in the measurement both the migration time of individual peaks and also their mass spectrum, facilitating the identification of degradation products (23).

Analysis of purity of paclitaxel

Paclitaxel ((2α,4α,5β,7β,10β,13α)-4,10-bis(acetyloxy)-13-{[(2R,3S)- 3-(benzoylamino)-2-hydroxy-3-phenylpropanoyl]oxy}-1,7-dihydroxy-9-oxo-5,20-epoxytax-11-en-2-yl benzoate) (Fig. 5) is derived from extracts of the Pacific Yew (*Taxus brevifolia*) (24). Due to the obtaining method, small amounts of other co-extracted taxanes may be found in medicines containing paclitaxel. Polish Pharmacopoeia VIII as a technique for the determination of paclitaxel impurities describes the LC (16).

Shao et al. presented another method - MEKC technique for the separation of paclitaxel from 12 related taxanes: 1) 10-deacetylbaccatin III, 2) baccatin III, 3) 10-deacetyl-7-xylosyltaxol B, 4) taxinine M, 5) 10-deacetyl-7-xylosyltaxol, 6) 10-deacetyl-7-xylosyltaxol C, 7) 7-xylosyltaxol, 8) 10-deacetyltaxol, 9) cephalomannine (taxol B), 10) 10-deacetyl-7-epi-taxol, 11) taxol C, 12) 7-epitaxol (25). An important factor influencing the separation efficiency proved to be SDS concentration. Increased concentration causes the formation of new micelles that interact with the substances dissolved in the basic solution, causing a lengthening of the migration time and increase resolution. There is also evidence that the absence of SDS prevents the separation of examined taxanes (25). Taxanes are highly hydrophobic molecules, having no charge, so in order to increase their solubility in the main buffer, organic solvent – ACN was added. With increasing concentration of ACN from 0% to 20%, migration time was elongated, and the separation was more effective. However, with increasing concentrations of ACN above 20% there was a significant reduction of the migration time. A higher increase in ACN concentration resulted in a gradual shortening of migration, and thus worse resolution (25). Optimal conditions to conduct separation were: 25 mM Tris buffer solution, 40 mM SDS, 30% ACN, 10 mM urea. Separation of thirteen taxanes conducted in this environment was the base for determination of impurities in the paclitaxel solution designed for intravenous injection, in which as a drug carrier polyethoxylated castor oil (Cremophor EL) was used (25). The migration direction of anionic micelles and thus the surfactant-taxane complex is opposite to the electroosmotic flow. Migration time of individual taxanes results from their decreasing solubility in the aqueous phase, and increasing binding to the micelles. Taxanes 1, 2, 5 do not have side carbon chains, which are responsible for the transfer of particles into the core of the micelle, thus their migration time is the shortest. The presence of xylose group increases solubility in the aqueous phase, resulting in migration time reduction, while the presence of an acetyl group instead of hydroxyl has the opposite effect on the migration of particles. When analyzing the purity of the paclitaxel solution in the polyetoxylated castor oil, it was showed that the additional peaks most likely originate from oligomers of Cremophor EL, what demonstrates the lack of impurities of other related taxanes (25).

CE was also used for the separation of different types of anti-cancer drugs – anthracyclines and taxanes simultaneously, which were separated by CZE, MEKC and MEEKC. The MEEKC method was characterized by very short separation time, high efficiencies of peaks and was proven to be basic for the separation of different combinations of anthracyclines and taxanes (26).

Analysis of KP46 purity

Tris(8-quinolinolato)gallium(III) (KP46) is a complex compound of gallium(III) in which the metal is linked to three ligands - molecules of 8-

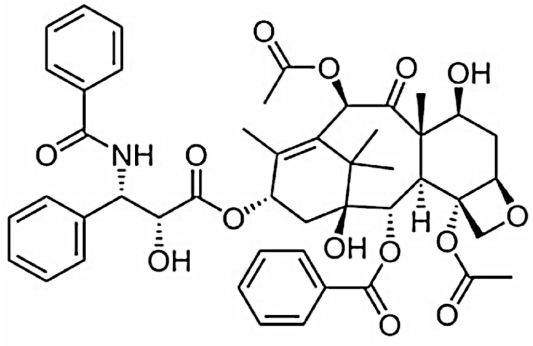

Figure 5. Structural formula of paclitaxel ((2α,4α,5β,7β,10β, 13α)-4,10-bis(acetyloxy)-13-{[(2R,3S)-3-(benzoylamino)-2-hydroxy-3-phenylpropanoyl]oxy}-1,7-dihydroxy-9-oxo-5,20-epoxytax-11-en-2-yl benzoate) (16)

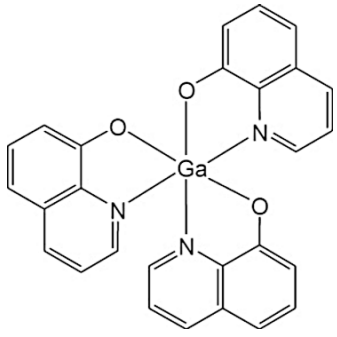

Figure 6. Structural formula of KP46 (tris(8-quinolinolato)gallium(III)) (28)

quinolinol (Fig. 6). Gallium's mechanisms of action include its binding to transferrin, targeting to transferrin receptors and inhibition of ribonucleotide reductase. Gallium activates caspases and induces apoptosis through the mitochondrial pathway (27). KP46 shows poor solubility in water and usually is administered orally in tablet form. The main impurity associated with the parent substance is 8-quinolinol (28).

Foteeva et al. analyzed the KP46 tablets by MEKC technique. MEKC analysis consisted of two basic steps: optimization of conditions for separation and sample preparation (28). The first step in optimizing the conditions for separation was to find the adequate concentration of SDS. With increasing concentration of SDS in the range 25-150 mM, lengthening of the migration time and the expansion of analyzed peaks was observed. Also, a decrease in resolution due to the distortion of the peaks was observed. SDS concentration equal to 50 mM provided a suitable resolution, short migration time and increase of KP46 signal. In order to further increase the resolution, 2-propanol was added to the solution in the amount of 25% (ACN and ethanol were less effective) (28).

Samples were injected hydrodynamically for 5 s at 20 mbar and separation was performed at a voltage of 15 kV, at 25^{o}C, at a wavelength of 210 nm. Length of silica capillary was 48 cm (40 cm to detector). The basic solution was 50 mM SDS in 10 mM phosphate buffer, pH 7.4, containing 25% of 2-propanol (23). Electropherogram of KP46 tablets obtained under these conditions has shown that the KP46 peak is clearly separated from the 8-quinolinol peak, what point out the effectiveness of this method (28). The possibility that the impurity exists in the concentration, which is not able to be detected by this method, was eliminated by the analysis by GC combined with MS (28). The same analytical strategy can be suggested for a similar evaluation of other metal-containing pharmaceuticals, as well as for profiling degradation-related impurities.

Advantages and disadvantages of capillary electrophoresis methods in the analysis of the drugs impurities

Number of publications concerning the analysis of the drugs purity by CE has been increased in recent years. The mechanism of particles separation is based on electroosmotic flow and electrokinetic current and is ideal for the separation of small molecules such as drugs. The advantages of CE include the multitude variations of analysis method. Wide choice of CE techniques: CZE, CGE, MEKC, MEEKC, CEC, CIEF, CITP, NACE allows adjusting the method to the chemical nature of the testing substance in order to obtain better results (11). Another advantage is the possibility of combining CE with multiple detection techniques such as spectrophotometry, mass spectrometry, light emitting diodes, fluorescence, and chemiluminescence (11). Electroosmotic flow and electrokinetic current may occur at the same time and may act in opposite directions, thus providing a high resolution. Small size of capillaries allows the use of small volume samples (4). The rate of separation is also an advantage. Depending on the length of the capillary, the basic separations can be done in minutes. Using a short length capillary and applying a high voltage, results can be obtained very quickly (7). CE is characterized by very low consumption of reagents, especially when compared with HPLC, what is associated with a decrease in the cost of analyses. In addition, the price comparison of silica capillaries and columns for HPLC is also in favor for CE. Time required for sample preparation is shorter compared to other analytical techniques. This applies to the determination of pure form of the drug, whereas the

Table 1. Comparison of separation techniques such as HPLC, CE and TLC (8).

	HPLC	**CE**	**TLC**
Sensitivity	+++	++	++
Precision at low concentrations	+++	++	+
Detection at low wavelength	+	+++	+
Cost of consumption	+	+++	+
Automation	+++	++	+
Analysis time (1-5 samples)	+	++	+
Analysis time (5-50 samples)	++	++	++
Selectivity	++	++	++

determination of drugs in biological fluids requires a more complex sample preparation (29).

However, there are also disadvantages of CE, which include the low limits of detection, especially in UV absorbance due to low optical path length, which is an usual detection technique in CE. For this reason, it is often necessary to use high concentration of samples or to do modification of the capillary. In the determination of impurities this needs particular attention because of the low concentrations of impurities that need to be detected. The solution to this problem is using the MS, which is the most accurate technique to identify unknown components found in the samples (29). Also, the ability to associate positively charged components of samples to the inner wall of the capillary, what could result in a decrease of method performance and change in analytes migration time, is a disadvantage (7). Another imperfection of CE is possibility of Joule heating generation, affecting the migration time, volume and surface of the resulting peaks. This problem was resolved by the use of cooling capillary solutions. Comparison of separation techniques: HPLC, TLC and CE has been shown in Table 1. With the rapid development, CE has become an analytical method exceeding TLC, in many aspects, but it is still not as popular as HPLC (8).

Distinct features of CE, such as very low sample consumption, minimal sample preparation, requirement of minimum of organic solvents, ease of buffer change and method development, speed of analysis, low cost of consumables, ensured its widespread applicability in clinical toxicology, therapeutic drug monitoring and forensic science, such as analysis of drugs in biologic specimens and various substances in food and environmental samples, including those encountered in clinical toxicology (30).

CONCLUSIONS

CE is a method which plays an important role in the analysis of the drugs impurities, including anti-cancer drugs, as was presented in this review. It is considered as an alternative to GC or HPLC. The technical development of equipment and accessories used in CE, the increasing number of publications concerning the separations by the CE, strengthen its position in pharmaceutical analysis. Further development of this method by increasing the speed and sensitivity of the separation can make CE a great tool in the pharmaceutical industry and facilitate the development and quality control of substances already in commerce, including anti-cancer drugs, whose quality control is particularly important due to its low therapeutic index. The applications of CE in several areas of pharmacy revealed that CE has shown real advantages over the various chromatographic methods and particularly in chiral drug analysis, protein/peptide and carbohydrate analysis.

REFERENCES

1. Gorog S.: Trends Anal. Chem. 25, 755 (2006).
2. Humfrey C.D.: Toxicol. Sci. 100, 24 (2007).
3. Handzlik J., Spengler G., Mastek B., Dela A., Molnar J., Amaral L., Kieć-Kononowicz K.: Acta Pol. Pharm. Drug Res. 69, 149 (2012).
4. Jorgenson J.W., Lukacs K.D.: Anal. Chem. 59, 1298 (1981).
5. Morzunova T.G.: Pharm. Chem. J., 40, 158 (2006).
6. Hilhorst M.J., Somsen G.W.: Electrophoresis, 22 2542 (2001).
7. Altria K.D., Elder D.: J. Chromatogr. A 1023 1 (2004).
8. Altria K.D.: J. Chromatogr. A 735, 43 (1996).
9. Mallampati, S., Pauwels, J., Hoogmartens, J., Van Schepdael, A.: CE in impurity profiling of drugs. in Capillary electrophoresis methods for pharmaceutical analysis, Ahuja S., Jimidar I. Eds., Chapt. 12, pp. 259-315, Elsevier, Academic Press, Amsterdam 2008.
10. Altria K., Marsh A., Sänger-van de Griend C.: Electrophoresis 27, 2263 (2006).
11. Suntornsuk L.: Anal. Bioanal. Chem. 398, 29 (2010).
12. Veuthey J.-L.: Anal. Bioanal. Chem, 38, 93 (2005).
13. Geiser L., Veuthey J.-L.: Electrophoresis, 28, 45 (2007).
14. ICH Harmonised Tripartite Guideline - Impurities in new drug substances Q3A (R2) (2006).
15. ICH Harmonised Tripartite Guideline - Impurities in new drug products Q3B (R2) (2006).
16. Polish Pharmacopoeia, VIII edn., Polish Pharmaceutical Society, Warszawa 2008.
17. Hartinger Ch.G., Keppler B.K.: Electrophoresis 28, 3436 (2007).
18. Verschraagen M., van der Born K., Zwiers T.H., van der Vijgh W.J.: J. Chromatogr. B 772, 273 (2002).
19. Wenclawiak B.W., Wollman M.: J. Chromatogr. A 724, 317 (1996).
20. Michalke B.: J. Trace Elem. Med. Biol. 24, 69 (2010).

21. Gotti R., Andrisano V.: Electrophoresis 25, 2830 (2004).
22. Pujol M., Girona V., Prat J., Muñoz M., De Bolós J.: Int. J. Pharm. 146, 263 (1997).
23. Li X.-F., Carter S. J., Dovichi N. J. : J. Chromatogr. A 895, 81 (2000).
24. Rao K.V.: Pharm. Res. 10, 521 (1993).
25. Shao L.K, Locke D.C.: Anal. Chem. 70, 897 (1998).
26. Shakalisava Y., Regan F.: Electrophoresis., 30, 3110 (2009).
27. Chitambar C.R.: Curr. Opin. Oncol. 16, 547 (2004).
28. Foteeva L.S., Stolyarova N.V., Timerbaev A.R., Keppler B.K.: J. Pharm. Biomed. Anal. 48, 218 (2008).
29. Hommerson P., Khan A.M.: Rapid Commun. Mass Spectrom., 23 2878 (2009).
30. Holzgrabe U., Brinz D., Kopec S., Weber C., Bitar Y.: Electrophoresis. 27, 2283 (2006).

Received: 14. 04. 2012