Journal of Pharmaceutical and Biomedical Analysis 55 (2011) 253–258

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Quality control of pharmaceutical formulations containing cisplatin, carboplatin, and oxaliplatin by micellar and microemulsion electrokinetic chromatography (MEKC, MEEKC)

Susanne Nussbaumer [a,b], Sandrine Fleury-Souverain [a,*], Julie Schappler [b], Serge Rudaz [b], Jean-Luc Veuthey [b], Pascal Bonnabry [a,b]

[a] Geneva University Hospitals (HUG), Pharmacy, Rue Gabrielle-Perret-Gentil 4, 1211 Geneva 14, Switzerland
[b] School of Pharmaceutical Sciences – EPGL, University of Geneva, University of Lausanne, 20 Bd d'Yvoy, 1211 Geneva 4, Switzerland

ARTICLE INFO

Article history:
Received 9 November 2010
Received in revised form 20 January 2011
Accepted 20 January 2011
Available online 28 January 2011

Keywords:
Anticancer drugs
Capillary electrophoresis
Platinum drugs
Micellar electrokinetic chromatography (MEKC)
Quality control
Validation

ABSTRACT

A micellar electrokinetic chromatography (MEKC) method was developed for the determination of cisplatin, carboplatin, and oxaliplatin in pharmaceutical formulations. The background electrolyte consisted of a phosphate buffer (pH 7.0; 25 mM) with sodium dodecyl sulfate (80 mM). The applied voltage was 30 kV and the sample injection was performed in the hydrodynamic mode. All analyses were carried out in a fused silica capillary with an internal diameter of 50 μm and a total length of 64.5 cm. The detection of target compounds was performed at 200 nm. Under these conditions, a complete separation of cisplatin, carboplatin and oxaliplatin was achieved in less than 10 min. The MEKC-UV method was validated and trueness values between 99.7% and 100.8% were obtained with repeatability and intermediate precision values of 0.7–1.4% and 1.1–1.7%, respectively for the three drugs. This method was found appropriate for controlling pharmaceutical formulations containing platinum complexes and successfully applied in quality control at the Geneva University Hospitals.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Platinum complexes belong to the most widely used drugs in cancer chemotherapy treatment and possess a pronounced activity in different cancer types by binding to the DNA and modifying its structure. Cisplatin, carboplatin, and oxaliplatin are the most important worldwide clinically approved platinum compounds (Fig. 1). Cisplatin was the first used platinum complex with a pronounced activity in testicular and ovarian cancers. The related analogs, carboplatin and oxaliplatin, were developed later to reduce the problematic side effects of cisplatin (nephrotoxicity, ototoxicity, peripheral neuropathy, etc.). Carboplatin is used in the treatment of advanced ovarian cancer and lung cancer, while oxaliplatin is licensed for the treatment of metastatic colorectal cancer in combination with fluorouracil and folinic acid [1].

Despite the use of platinum compounds for several decades, there are only few published analytical methods. As reported in the review by Espinosa Bosch et al., different techniques were developed for the determination of cisplatin, such as derivative spectrophotometry, phosphorescence, atomic absorption spectrometry, gas chromatography, capillary electrophoresis (CE) and high performance liquid chromatography (HPLC) coupled with different detectors (UV–vis, electrochemical, inductively coupled plasma–atomic emission spectrometry, inductively coupled plasma–mass spectrometry (ICP–MS) or electrospray ionization–mass spectrometry) [2]. Regarding carboplatin and oxaliplatin, no specific reviews about analytical methods have been published to the author's knowledge. Most common techniques for these compounds are HPLC or CE coupled to UV–vis or MS detection. During the last years, ICP–MS has become very popular for the determination of the three platinum compounds in environmental, biological, and clinical samples [2].

According to Hartinger et al., CE has emerged as the method of choice for the separation of intact platinum metal complexes and their metabolites due to its high efficiency, versatility and gentle separation conditions for metal complexes [3–5]. Analysis of anticancer drugs by CE appears to be very interesting due to the toxicity of these compounds, because the separation is performed in a closed system and the waste volume is on the μL range.

The three tested platinum drugs are non-charged coordination complexes. Therefore, simple CZE is not adapted for resolving these compounds and other separation techniques are necessary, such as micellar electrokinetic chromatography (MEKC) or microemulsion electrokinetic chromatography (MEEKC). In MEKC, an ionic surfac-

* Corresponding author. Tel.: +41 22 382 39 78; fax: +41 22 382 39 65.
E-mail address: sandrine.fleury.souverain@hcuge.ch (S. Fleury-Souverain).

0731-7085/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2011.01.029

tant, generally sodium dodecyl sulfate (SDS), is added to the BGE at a higher concentration than its critical micelle concentration and micelles act as pseudo-stationary phase allowing solute partition simultaneously to electrophoretic process [6]. MEEKC has a similar operating principle except that a microemulsion (ME) is used. As reported in several studies, MEEKC may present advantages over MEKC such as enhanced solubilization power and an enlarged migration window [7,8].

The main publications dedicated to the analysis of platinum drugs with MEKC or MEEKC were developed for biological studies, such as clinical sample analysis [9], drug–protein [10–14] and drug–DNA (or nucleotides) binding studies [15–19] or for chemical studies [20,21]. Usually, UV spectrophotometry was used for the detection of platinum drugs with MEKC or MEEKC even if ICP–MS was also reported to enhance selectivity and sensitivity [22].

For quality control of pharmaceutical formulations, UV detection was found sufficient in terms of sensitivity because the limit of quantification of platinum compounds was inferior to their concentration in drug products. To our knowledge, only one MEKC method has been reported in the literature for quality control of platinum formulations and no complete validation was performed [23].

At the pharmacy of the Geneva University Hospitals (HUG), more than 20% of prepared chemotherapies are platinum formulations (including cisplatin, carboplatin, and oxaliplatin). The role of the hospital pharmacy is to dilute or dissolve commercially available pharmaceutical formulations in appropriate conditions to ensure the protection of nurses and the sterility of the injectable solution. For the quality control of such reconstituted formulations, a method for the determination of these compounds is necessary.

The objective of this study was to develop and validate a simple MEEKC or MEKC–UV method to determine cisplatin, carboplatin, and oxaliplatin in pharmaceutical formulations and to apply it in quality control.

2. Experimental

2.1. Chemicals

The study was performed with the following commercially available cytotoxic drugs (see Fig. 1): Cisplatin Ebewe® 1 mg mL^{-1} was purchased from Sandoz Pharmaceuticals SA (Steinhausen, Switzerland) and Carboplatin Teva® 10 mg mL^{-1} from Teva Pharma AG (Aesch, Switzerland). Eloxatin® (containing oxaliplatin, 50 mg) was obtained from Sanofi-Aventis (Meyrin, Switzerland) and reconstituted with glucose 5% from Sintetica-Bioren SA (Couvet, Switzerland) to obtain a final concentration of 5 mg mL^{-1}.

Caffeine citrate used as internal standard (IS) was purchased from Fagron GmbH (Barsbüttel, Germany).

Concentrated phosphoric acid and NaOH 1 M were obtained from Merck (Darmstadt, Germany), sodium dodecyl sulfate (SDS) from Fluka (Buchs, Switzerland) and ultra-pure water was supplied by a Milli-Q Plus unit from Millipore (Bedford, MA, USA). n-heptane was purchased from Merck (Darmstadt, Germany), n-octane and

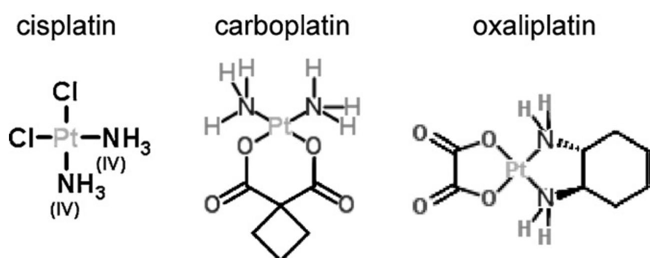

Fig. 1. Structures of cisplatin, carboplatin and oxaliplatin.

n-butanol from Fluka (Buchs, Switzerland). Ceofix® kit was from Analis (Suarlée, Belgium).

Water for injection and NaCl 0.9% used in the preparation of pharmaceutical formulations were obtained by Bichsel laboratories (Interlaken, Switzerland) and glucose 5% was from Sintetica-Bioren SA (Couvet, Switzerland).

2.2. BGE preparation

For MEEKC, different microemulsions (ME) were prepared from a 20 mM phosphate buffer set at pH 2.0, 7.0 and 10 mM borate buffer set at pH 9.0. Different ratios of SDS, n-butanol and n-octane or n-heptane were tested: 6.6% (w/v) n-butanol, 3.3% (w/v) SDS, and 0.78% (w/v) n-heptane; 7.3% (w/v) n-butanol, 2.3% (w/v) SDS, and 0.82% (w/v) n-octane; 6.6% (w/v); n-butanol, 3.3% (w/v) SDS, and 0.78% (w/v) n-octane. SDS was partially dissolved in approximately 80% of the buffer before adding n-butanol and n-heptane. The mixture was then carefully shaken until SDS was completely dissolved, and the remaining buffer added. The solution was left to stand for 1 h at room temperature. Before use, the ME was filtered through a 0.45 μm microfilter (BGB Analytik, Böckten, Switzerland). The ME was stored at room temperature and remained stable for at least one week.

For MEKC, different BGEs were tested: borate (pH 9.2; 50 mM) with SDS (80 mM); acetate (pH 4.75; 50 mM) with SDS (80 mM), phosphate (pH 7.0) with different buffer concentration (10, 25 and 50 mM) and SDS concentration (30, 60 and 80 mM). The final composition consisted of 25 mM phosphate at pH 7.0 with 80 mM SDS. The aqueous BGE was prepared by an adequate dilution of the concentrated acidic solution, and a volume of NaOH 1 M was added to adjust the solution at pH 7.0. The solution was then diluted to the final volume with water and SDS dissolved to obtain a final concentration of 80 mM. The BGE was degassed in an ultrasonic bath for 10 min before use.

2.3. Instrumentation

CE experiments were carried out with an HP^{3D}CE system (Agilent Technologies, Waldbronn, Germany) equipped with a diode-array detector, an autosampler and a power supply able to deliver up to 30 kV. A CE ChemStation (Agilent) was used for CE control, data acquisition and data handling.

Analyses were performed in uncoated fused silica (FS) capillaries from BGB Analytik AG (Böckten, Switzerland) with an internal diameter (i.d.) of 50 μm, an outside diameter (o.d.) of 375 μm and a total length of 64.5 cm (effective length of 56.5 cm).

The capillary was thermostated at 25 °C in a high velocity air stream and a voltage of 30 kV was applied in the positive mode. The generated current was between 20 and 70 μA depending on the BGE. Samples were kept at ambient temperature in the autosampler and injected in the hydrodynamic mode to fill approximately 1% of the effective capillary length (40 mbar for 10 s). The detection was achieved at 200 nm with a band width of 10 nm and a response time of 0.1 s.

Before first use, FS capillaries were sequentially rinsed with methanol, 0.1 M NaOH, water, methanol, 0.1 M HCl, water and BGE for 5 min. Prior to each sample injection, the capillary was rinsed by pressure (940 mbar) for 3 min with fresh BGE ensuring good repeatability of migration times. When not in use, the capillary was washed with water and methanol. As the electrophoresis process altered the running buffer pH by electrolysis, the separation buffer was refreshed every six runs at the inlet and outlet vials.

For MEEKC performed at pH 2, the capillary was coated with Ceofix® according to the publication of Henchoz et al. [24] to ensure a high EOF at low pH. Before an analytical series, several wash-

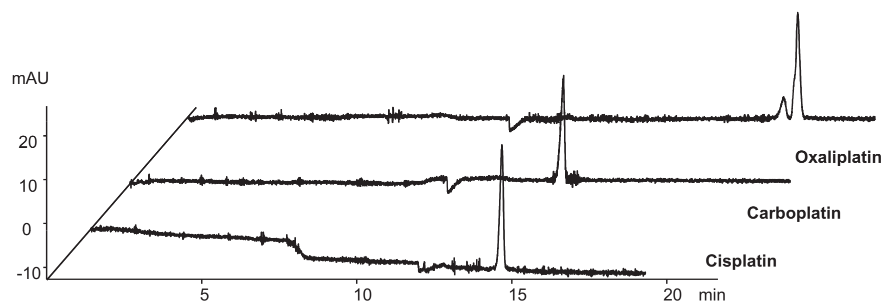

Fig. 2. Electropherogram obtained for the MEEKC–UV analysis of standard samples containing cisplatin, carboplatin, and oxaliplatin at 0.5 mg mL^{-1} in an aqueous solution. BGE: 20 mM phosphate at pH 7.0 with 6.6% (w/v) n-butanol, 3.3% (w/v) SDS, and 0.78% (w/v) n-heptane. Voltage: 20 kV. All other experimental conditions are described in Section 2.3.

ing steps (1 bar) were carried out: water (1 min), Ceofix® initiator (1 min), Ceofix® accelerator (1 min), BGE (5 min), and then the separation voltage (20 kV) was applied for 5 min. Prior to each sample injection (preconditioning step), the capillary was rinsed (1 bar) with BGE for 3 min. No postconditioning was performed.

2.4. Method validation

A validation was performed to estimate quantitative parameters of the method for the analysis of cisplatin, carboplatin, and oxaliplatin in pharmaceutical formulations. The validation was based on ICH guidelines following the recommendations of the Société Française des Sciences et Techniques Pharmaceutiques (SFSTP) [25]. Quantitative performance was estimated in three separate series ($j = 3$) with the V2 protocol. This protocol involves three concentration levels ($k = 3$) with two repetitions ($n = 2$) for calibration standards (CS) and three concentration levels ($k = 3$) with four repetitions ($n = 4$) for validation standards (VS). Each series involved the injection of a freshly prepared BGE, complete washing of the capillary with water and methanol, and instrument shut-off. Caffeine citrate was used as internal standard (IS). Calculations were performed using normalized area (area/migration time) ratios of the three platinum drugs on the internal standard.

2.5. Sample preparation

All solutions were prepared in appropriate conditions for handling hazardous compounds as cytotoxic agents. Moreover, the development of the method was performed with drug specialities to avoid direct contact of the operator to cytotoxic powder and to minimize contamination risk by preparing working solutions. For the validation, standard solutions of cisplatin and oxaliplatin were compared with pharmacopeia reference standards and no difference between the electropherograms was observed (data not shown). Therefore, the validation was also performed with drug specialities.

CS and VS were independently prepared for each platinum compound. For stability reasons and to avoid drug interactions, the three platinum complexes were separately analysed. Cisplatin was prepared in NaCl 0.9% to avoid hydrolysis, while carboplatin is modified to cisplatin in presence of chloride. The IS stock solution was prepared by dissolving caffeine citrate in ultra-pure water at a concentration of 1.0 mg mL^{-1}. CS and VS were stable for at least three days at 25 °C and no degradation was observed during the analysis.

2.5.1. Calibration standard

For CS, three concentration levels at 0.05, 0.5, and 1 mg mL^{-1} of cisplatin, carboplatin, and oxaliplatin were prepared by diluting the appropriate volume of drug specialities in water. 50 μL of caffeine citrate at 1 mg mL^{-1} (IS) was added to 500 μL of the prepared solutions.

2.5.2. Validation standard

For VS, three concentration levels at 0.05, 0.5, and 1 mg mL^{-1} of cisplatin, carboplatin, and oxaliplatin were prepared by diluting the appropriate volume of drug specialities in NaCl 0.9% for cisplatin and glucose 5% for carboplatin and oxaliplatin. 50 μL of caffeine citrate at 1 mg mL^{-1} (IS) was added to 500 μL of the prepared sample.

2.6. Application to pharmaceutical formulations

Cisplatin, carboplatin, and oxaliplatin were determined in pharmaceutical formulations prepared by the HUG pharmacy. The formulations were diluted in distilled water to obtain a final concentration between 0.05 and 1 mg mL^{-1} of the platinum compound. Quantitative analyses were repeated in duplicate for each formulation.

3. Results and discussion

3.1. Method development

3.1.1. Microemulsion electrokinetic chromatography (MEEKC)

To ensure the identity of the platinum compound in formulations, separation of the three drugs was mandatory. Different MEs were tested: 20 mM phosphate at pH 2.0 and pH 7.0, 20 mM borate at pH 9.0, with different ratios of SDS, n-butanol, n-octane and n-heptane, respectively. Among the tested experimental conditions, best separation of the three platinum compounds was obtained with a phosphate ME at 20 mM and pH 7.0, 6.6% (w/v) n-butanol, 3.3% (w/v) SDS, and 0.78% (w/v) n-heptane and an applied voltage of 20 kV (Fig. 2). Analysis time was long (20 min), but high resolution between the three compounds (>7) and good efficiency was obtained ($N > 70{,}000$). With the phosphate ME at pH 2.0, resolution between cisplatin and carboplatin was lower ($Rs \sim 2$) and analysis time was inferior to 10 min for all compounds due to Ceofix® coating. Similar efficiency was obtained for all compounds ($N > 70{,}000$). With the borate ME at pH 9.0, also good separation was obtained, but a better stability of platinum complexes was observed at lower pH [4]. The tested ratios of SDS, n-heptane, n-octane and n-butanol did not influence the separation significantly (data not shown).

For oxaliplatin, two peaks were observed in all selected conditions. The experiments were performed with the commercially available Eloxatin and the second peak was supposed to be an additive or impurity present in the formulation. Therefore, the analysis was repeated with a Pharmacopeia Reference Standard of oxaliplatin and with the Pharmacopeia Impurities A, B, C and D. The same electropherogram was obtained with the Reference Standard as with Eloxatin. Impurity A was not detected in the separation

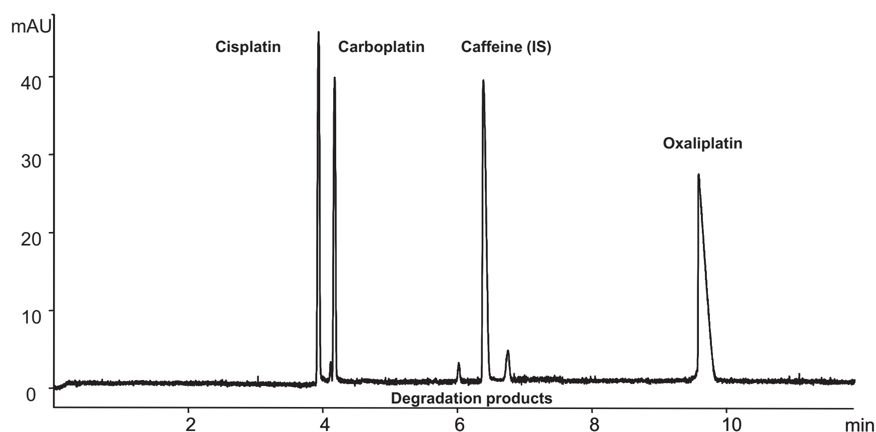

Fig. 3. Electropherograms obtained for the MEKC–UV analysis of a standard sample containing cisplatin, carboplatin, and oxaliplatin at 0.5 mg mL^{-1} with caffeine (IS) at 0.1 mg mL^{-1} in an aqueous solution. BGE: 25 mM phosphate at pH 7.0 with SDS 80 mM. All other experimental conditions are described in Section 2.3.

window. Impurities B and C were completely separated from oxaliplatin and did not migrate with the unknown peak. No resolution between oxaliplatin and the Impurity D, corresponding to the S–S enantiomer, was observed (data not shown). Thus, these experiments demonstrated that the second peak observed for oxaliplatin was not due to impurities or additives present in the formulation.

To exclude a degradation of oxaliplatin due to the separation conditions (20 kV, 25 °C), instrument parameters were modified and different conditions of voltage (15 kV and 30 kV) and temperature (15 °C and 35 °C) were applied. However, no difference was obtained for the second peak of oxaliplatin (data not shown). MEs were also prepared with solvents from different origins, to exclude a reaction between oxaliplatin and an impurity in the BGE system. With all tested MEs, both peaks for oxaliplatin were observed.

Some hypotheses found in the literature could explain this behavior. Oxaliplatin possesses a 1,2-diaminocyclohexane (DACH) carrier ligand and according to Tyagi et al. [26], several conformers coexist at room temperature, which might explain the presence of a second peak for oxaliplatin with MEEKC. Another study reported intramolecular transformations of platinum complexes with aminoalcohol ligand and the possibility of separating singly ring-opened and doubly ring-opened species. This apparently takes place due to the shifting of the equilibrium toward the ring-opened species induced by adduct formation between SDS and the platinum complex [27]. But to our knowledge, this behavior has never been reported for oxaliplatin. Moreover, in the following experiments with MEKC, only one peak was observed for oxaliplatin.

Another problem of the MEEKC method might be the quantification of platinum drugs in very low concentrated formulations, especially for preparations containing cisplatin, because of insufficient sensitivity. Given the presence of two peaks for the analysis of oxaliplatin and the limited sensitivity, an alternative strategy based on MEKC was investigated to perform the quality control of platinum drugs in hospital formulations.

3.1.2. *Micellar electrokinetic chromatography (MEKC)*

Different BGEs were tested including borate, phosphate and acetate buffer at different concentrations, pH and SDS concentrations. At increased pH value, platinum complexes can be hydrolyzed [4] and therefore, the borate BGE (pH 9.2) was excluded. However, with acetate BGE (pH 4.5) cisplatin was co-migrating with the EOF. Finally, a phosphate buffer (pH 7.0) was chosen as compromise and no degradation was observed during the analysis.

The first analyses were performed with a buffer concentration of 10 mM. Nevertheless, to improve the resolution between cisplatin, carboplatin, oxaliplatin and EOF, buffer solutions with different molarities (25, 50 mM) and different SDS concentrations (30, 60, 80 mM) were studied. Among the tested BGEs, best separation was obtained with 25 mM phosphate and 80 mM SDS. Analysis time was inferior to 10 min and acceptable resolution (Rs > 4) and efficiency ($N \sim 70{,}000$) was obtained for all compounds. Under these conditions the generated current was still acceptable (~50 μA). With higher SDS amount, the generated current was too high and capillary breakdown was observed.

The separation between the three platinum drugs was also studied in presence of 5, 10 and 20% of acetonitrile. As reported, solvent modified MEKC could sometimes achieve better separation conditions [28,29]. For oxaliplatin, the migration time decreased with increased ACN concentration. But the resolution between cisplatin and carboplatin was also lowered with ACN (data not shown). Therefore, a purely aqueous phosphate BGE (pH 7.0; 25 mM) containing 80 mM of SDS was selected (Fig. 3).

Comparing to MEEKC, the selected MEKC method presented similar efficiency and shorter analysis time. Moreover, better sensitivity allowed the analysis of low concentrated formulations. Therefore, the MEKC method was selected for quality control of pharmaceutical formulation and a complete validation was performed.

3.2. *Method validation*

The concentrations of the prescribed platinum drugs at HUG were considered for the determination of the concentration range used in the validation. For cisplatin, concentrations between 0.05 and 0.4 mg mL^{-1} (median: 0.16 mg mL^{-1}), for carboplatin 0.1 and 2.5 mg mL^{-1} (median: 1.4 mg mL^{-1}) and for oxaliplatin 0.1 and 1.0 mg mL^{-1} (median: 0.4 mg mL^{-1}) were prescribed in 2009. In order to decrease the number of manipulations with toxic compounds, formulations were injected with simple or without dilution. Therefore, the concentration range was fixed from 0.05 to 1 mg mL^{-1} for all three compounds. Caffeine citrate chosen as IS was detected between carboplatin and oxaliplatin.

The calibration curve was obtained for each series with conventional least-squared linear regression using the three concentration levels (0.05 mg mL^{-1}, 0.5 mg mL^{-1} and 1.0 mg mL^{-1}). After establishing the calibration curves for each series, concentrations of VS were computed from the analytical response to obtain trueness, repeatability and intermediate precision. Trueness was expressed (in percentage) as the ratio between theoretical and average measured values at each concentration level. Repeatability and intermediate precision were expressed as the relative standard deviation (RSD%), i.e., the ratio of the intra-day standard deviation (s_r) and between-day standard deviation (s_R), respectively,

Table 1
Validation results: trueness, repeatability and intermediate precision of the developed MEKC–UV method for the analysis of cisplatin, carboplatin and oxaliplatin in pharmaceutical formulations.

Theoretical concentration [mg mL^{-1}]	Trueness	Repeatability (RSD)	Intermediate precision (RSD)
Cisplatin			
0.05	100.6%	1.0%	1.7%
0.5	100.1%	0.7%	1.4%
1	100.8%	1.1%	1.1%
Carboplatin			
0.05	100.7%	0.8%	1.6%
0.5	100.0%	0.7%	1.4%
1	99.7%	0.7%	1.3%
Oxaliplatin			
0.05	100.2%	1.4%	1.4%
0.5	99.9%	0.9%	1.3%
1	100.3%	1.3%	1.4%

on the theoretical concentrations [30]. The s_r and s_R values were obtained using ANOVA analysis. As reported in Table 1, trueness and precision values were in accordance with recommendations for the analysis of pharmaceutical formulations over the tested concentration range. The RDS (repeatability and intermediate precision) was lower than 2%, with trueness values between 99.7 and 100.8%. To visualize the overall method variability, the accuracy profile was built combining trueness and intermediate precision as the confidence interval [31]. As presented in Fig. 4, the total error did not exceed acceptance limits (±5%) for all concentration levels. Consequently, the developed MEKC–UV method could be considered accurate for the three platinum drugs over the tested range.

3.3. Application to pharmaceutical formulations

In order to demonstrate the applicability of the MEKC–UV method to real samples, determination of the three platinum drugs was achieved in pharmaceutical formulations for quality control. The concentrations of the cytotoxic agents were calculated with reference to a calibration curve constructed the same day. CS at three concentration levels were replicated twice, and conventional least-squared linear regression was applied. Since two independent analyses ($N = 2$) were performed on each pharmaceutical formulation, the result of the analysis could be expressed as:

$$\mathrm{cnf}(x) = \bar{x} \pm t_{\mathrm{df},\alpha}\sqrt{\frac{s_r^2}{N} + s_g^2} \quad (1)$$

where N is the number of analyses performed and $\bar{x}$ is the mean result. The $t_{\mathrm{df},\alpha}$ (Student's constant depending on df and α set at 5%), s_r^2 and s_g^2 variance values were determined during the validation step with the regular ANOVA-based variance decomposition [31]. The analysis repetition was useful to obtain a smaller confidence interval, since most of the variability came from repeatability (s_r^2). As shown in Table 2, prescribed concentrations of pharmaceutical formulations were confirmed to be in the range of ±10% of the target value by the MEKC–UV method, which corresponds to the acceptance limits for these formulations at the HUG pharmacy.

Table 2
Analysis of the three cytotoxic drugs by MEKC–UV in pharmaceutical formulations prepared at the HUG pharmacy.

Batch number	Concentration
CYT/10-123162 104 mg cisplatin in 604 mL NaCl 0.9%	107.0 ± 2.8%
CYT/10-122999 140 mg cisplatin in 640 mL NaCl 0.9%	100.8 ± 2.8%
CYT/10-121694 40 mg cisplatin in 540 mL NaCl 0.9%	106.9 ± 2.8%
CYT/10-122599 529 mg carboplatin in 303 mL glucose 5%	96.8 ± 2.8%
CYT/10-122482 260 mg oxaliplatin in 302 mL glucose 5%	95.0 ± 2.0%
CYT/10-122846 114 mg oxaliplatin in 273 mL glucose 5%	94.3 ± 2.0%
CYT/10-123120 120 mg oxaliplatin in 274 mL glucose 5%	97.1 ± 2.0%

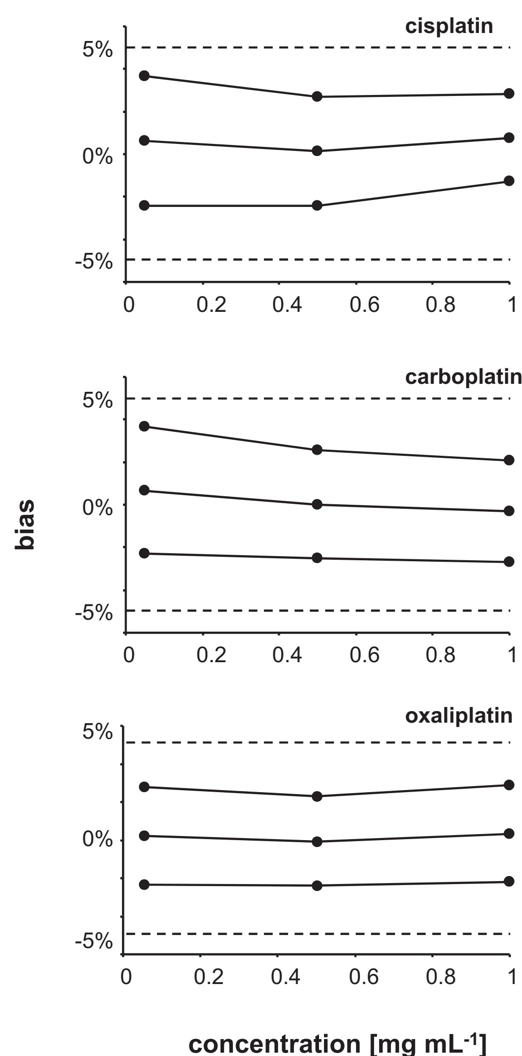

Fig. 4. Accuracy profiles of the developed MEKC–UV method for the determination of cisplatin, carboplatin, and oxaliplatin in a pharmaceutical formulation using a linear regression model. The dashed lines represent the acceptance limits of ±5%.

4. Conclusions

Different methods based on MEKC and MEEKC were developed for the quantitative determination of cisplatin, carboplatin, and oxaliplatin in pharmaceutical formulations. The MEKC method exhibited very good quantitative performance in terms of accuracy and precision with an analysis time of less than 10 min for the three platinum compounds. The manipulation steps, including the handling of cytotoxic agents, are reduced to dilution and addition of the IS to the pharmaceutical formulation. Therefore, the presented MEKC–UV method can be used as a very simple technique in quality control and was successfully applied in routine analysis at HUG pharmacy.

References

[1] D.E. Thurston, Chemistry and Pharmacology of Anticancer Drugs, CRC Press Taylor and Francis Group, Boca Raton, 2007.
[2] M. Espinosa Bosch, A.J. Ruiz Sánchez, F. Sánchez Rojas, C. Bosch Ojeda, Analytical methodologies for the determination of cisplatin, J. Pharm. Biomed. Anal. 47 (2008) 451–459.

[3] A.R. Timerbaev, A. Küng, B.K. Keppler, Capillary electrophoresis of platinum-group elements: analytical, speciation and biochemical studies, J. Chromatogr. A 945 (2002) 25–44.

[4] C.G. Hartinger, A.R. Timerbaev, B.K. Keppler, Capillary electrophoresis in anticancer metallodrug research: advances and future challenges, Electrophoresis 24 (2003) 2023–2037.

[5] C.G. Hartinger, B.K. Keppler, CE in anticancer metallodrug research – an update, Electrophoresis 28 (2007) 3436–3446.

[6] S. Terabe, Micellar electrokinetic chromatography for high-performance analytical separation, Chem. Rec. 8 (2008) 291–301.

[7] J. Schappler, D. Guillarme, J. Prat, J.-L. Veuthey, S. Rudaz, Coupling CE with atmospheric pressure photoionization MS for pharmaceutical basic compounds: optimization of operating parameters, Electrophoresis 28 (2007) 3078–3087.

[8] R. Ryan, S. Donegan, J. Power, K. Altria, Advances in the theory and application of MEEKC, Electrophoresis 31 (2010) 755–767.

[9] Z. Huang, A.R. Timerbaev, B.K. Keppler, T. Hirokawa, Determination of cisplatin and its hydrolytic metabolite in human serum by capillary electrophoresis techniques, J. Chromatogr. A 1106 (2006) 75–79.

[10] A.R. Timerbaev, C.G. Hartinger, S.S. Aleksenko, B.K. Keppler, Interactions of antitumor metallodrugs with serum proteins: advances in characterization using modern analytical methodology, Chem. Rev. 106 (2006) 2224–2248.

[11] A.V. Rudnev, S.S. Aleksenko, O. Semenova, C.G. Hartinger, A.R. Timerbaev, B.K. Keppler, Determination of binding constants and stoichiometries for platinum anticancer drugs and serum transport proteins by capillary electrophoresis using the Hummel–Dreyer method, J. Sep. Sci. 28 (2005) 121–127.

[12] M. Groessl, C.G. Hartinger, K. Polec-Pawlak, M. Jarosz, B.K. Keppler, Capillary electrophoresis hyphenated to inductively coupled plasma–mass spectrometry: a novel approach for the analysis of anticancer metallodrugs in human serum and plasma, Electrophoresis 29 (2008) 2224–2232.

[13] T. Lemma, J. Pawliszyn, Human serum albumin interaction with oxaliplatin studied by capillary isoelectric focusing with the whole column imaging detection and spectroscopic method, J. Pharm. Biomed. Anal. 50 (2009) 570–575.

[14] A.R. Timerbaev, S.S. Aleksenko, K. Polec-Pawlak, R. Ruzik, O. Semenova, C.G. Hartinger, S. Oszwaldowski, M. Galanski, M. Jarosz, B.K. Keppler, Platinum metallodrug-protein binding studies by capillary electrophoresis–inductively coupled plasma–mass spectrometry: characterization of interactions between Pt(II) complexes and human serum albumin, Electrophoresis 25 (2004) 1988–1995.

[15] A. Küng, A. Zenker, M. Galanski, B.K. Keppler, Capillary electrophoretic study of carboplatin and analogues with nucleoside monophosphates, di- and trinucleotides, J. Inorg. Biochem. 83 (2001) 181–186.

[16] A. Zenker, M. Galanski, T.L. Bereuter, B.K. Keppler, W. Lindner, Capillary electrophoretic study of cisplatin interaction with nucleoside monophosphates, di- and trinucleotides, J. Chromatogr. A 852 (1999) 337–346.

[17] A. Zenker, M. Galanski, T.L. Bereuter, B.K. Keppler, W. Lindner, Kinetics of binding properties of 5′-GMP with cisplatin under simulated physiological conditions by capillary electrophoresis, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 745 (2000) 211–219.

[18] C.G. Hartinger, P. Schluga, M. Galanski, C. Baumgartner, A.R. Timerbaev, B.K. Keppler, Tumor-inhibiting platinum(II) complexes with aminoalcohol ligands: comparison of the mode of action by capillary electrophoresis and electrospray ionization–mass spectrometry, Electrophoresis 24 (2003) 2038–2044.

[19] U. Warnke, C. Rappel, H. Meier, C. Kloft, M. Galanski, G. Hartinger Christian, K. Keppler Bernhard, U. Jaehde, Analysis of platinum adducts with DNA nucleotides and nucleosides by capillary electrophoresis coupled to ESI–MS: indications of guanosine 5′-monophosphate O_6–N_7 chelation, Chembiochem 5 (2004) 1543–1549.

[20] S. Oszwaldowski, A.R. Timerbaev, Development of quantitative structure–activity relationships for interpretation of the migration behavior of neutral platinum(II) complexes in microemulsion electrokinetic chromatography, J. Chromatogr. A 1146 (2007) 258–263.

[21] C. Rappel, M. Galanski, A. Yasemi, L. Habala, B.K. Keppler, Analysis of anticancer platinum(II)-complexes by microemulsion electrokinetic chromatography: separation of diastereomers and estimation of octanol–water partition coefficients, Electrophoresis 26 (2005) 878–884.

[22] A.K. Bytzek, M.R. Reithofer, M. Galanski, M. Groessl, B.K. Keppler, C.G. Hartinger, The first example of MEEKC–ICP–MS coupling and its application for the analysis of anticancer platinum complexes, Electrophoresis 31 (2010) 1144–1150.

[23] B.W. Wenclawiak, M. Wollmann, Separation of platinum(II) anti-tumour drugs by micellar electrokinetic capillary chromatography, J. Chromatogr. A 724 (1996) 317–326.

[24] Y. Henchoz, S. Romand, J. Schappler, S. Rudaz, J.-L. Veuthey, P.-A. Carrupt, High-throughput log P determination by MEEKC coupled with UV and MS detections, Electrophoresis 31 (2010) 952–964.

[25] P. Hubert, J.J. Nguyen-Huu, B. Boulanger, E. Chapuzet, P. Chiap, N. Cohen, P.A. Compagnon, W. Dewe, M. Feinberg, M. Lallier, M. Laurentie, N. Mercier, G. Muzard, C. Nivet, L. Valat, Validation des procédures analytiques quantitatives – harmonisation des démarches, STP Pharma Pratiques 13 (2003) 101–138.

[26] P. Tyagi, P. Gahlot, R. Kakkar, Structural aspects of the anti-cancer drug oxaliplatin: a combined theoretical and experimental study, Polyhedron 27 (2008) 3567–3574.

[27] P. Schluga, C.G. Hartinger, M. Galanski, K. Meelich, A.R. Timerbaev, B.K. Keppler, Tumour-inhibiting platinum (II) complexes with aminoalcohol ligands: biologically important transformations studied by micellar electrokinetic chromatography, nuclear magnetic resonance spectroscopy and mass spectrometry, Analyst 130 (2005) 1383–1389.

[28] S Cherkaoui, L. Mateus, P. Christen, J. -L. Veuthey, Micellar electrokinetic capillary chromatography for selected tropane alkaloid analysis in plant extract, Chromatographia 46 (1997) 351–357.

[29] K. Ortner, W. Buchberger, M. Himmelsbach, Capillary electrokinetic chromatography of insulin and related synthetic analogues, J. Chromatogr. A 1216 (2009) 2953–2957.

[30] E. Rozet, A. Ceccato, C. Hubert, E. Ziemons, R. Oprean, S. Rudaz, B. Boulanger, P. Hubert, Analysis of recent pharmaceutical regulatory documents on analytical method validation, J. Chromatogr. A 1158 (2007) 111–125.

[31] L. Geiser, S. Rudaz, J.-L. Veuthey, Decreasing analysis time in capillary electrophoresis: validation and comparison of quantitative performances in several approaches, Electrophoresis 26 (2005) 2293–2302.