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Short communication

Capillary electrophoretic assay for the stability of tris(8-quinolinolato)gallium(III) in tablet formulations

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ABSTRACT

A capillary electrophoresis (CE) method for testing the stability of a novel oral anticancer metallodrug, tris(8-quinolinolato)gallium(III) (KP46), is proposed. As both the intact drug and its eventual impurity or/and decomposition product, 8-quinolinol, are not charged (at most of the pH range), the micellar-mediated CE mode based on using micellar concentrations of sodium dodecyl sulfate was employed. The running electrolyte conditions were optimized in order to resolve the peak of KP46 from the signal of 8-quinolinol, as well as from these of tablet matrix components. The stability of KP46 in different organic and water-organic solvent systems was studied regarding its limited solubility and the following recovering experiments. The method thus developed was applied to the determination of KP46 in tablet formulations, for which sample preparation method, namely powdering and ultrasound-assisted extraction (with 50% aqueous acetone), was tested and optimized in terms of procedure time (10 min). Different in the content of the active substance (10–30%) batches of tablets stored for two years after preparation were validated and recoveries obtained at the level from 97 to 102% confirmed sufficient drug stability. This principal finding was verified by means of an independent method, gas chromatography coupled with mass spectrometry (GC–MS).

1. Introduction

Metal compounds, including metal complexes, are recognized as important therapeutic drugs [1]. Particularly significant roles metal-based drugs play in contemporary chemotherapy where platinum(II) tumor-inhibiting agents are applied in 50–70% of all cancer patients [2]. Substantial efforts devoted to the discovery of anticancer drugs with greater efficacy and reduced toxic side effects resulted in developing a number of potent non-platinum agents [3,4]. Among these, a gallium(III) investigational compound, tris(8-quinolinolato)gallium(III) (KP46), was found to prevent the in vivo proliferation of cancer cells and exert the apoptosis-inducing effects and considerable antihypercalcemic properties [5,6]. This has recently been demonstrated in human clinical trials [7]. Because of its limited aqueous solubility KP46 is orally administrated, typically in the form of tablets. Assaying of KP46 and degradation-related impurities in drug substance and pharmaceutical preparations appears important for estimating the side-effect profile of the drug and establishing shelf-life and storage conditions.

Pharmaceutical analysis became one of the most productive areas where the use of capillary electrophoresis (CE) methods has proved particularly successful [8,9]. The wide range of pertinent CE applications encompasses determination of pharmaceutical content and excipients, impurity profiling, chiral analysis, and measurement of drug physicochemical properties (pK_a, $\log P$). For analyses of neutral and/or water-insoluble drugs as well as their mixtures with acidic and basic pharmaceutical compounds, CE can be beneficially applied in micellar or microemulsion electrokinetic chromatography modes [10,11].

In this paper, a CE method is introduced for KP46 and the major drug-related impurity, 8-quinolinol (HQ), in bulk drug and formulated products. The stability analysis of KP46 in tablets requires a key sample preparation step, which has also been an objective of this research. The experimental conditions were independently optimized for variables influencing the target analyte resolution and those affecting recovery of the drug from solid dosage form. The results of CE analysis within various tablet batches, proving

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satisfactory stability of KP46, will also be presented and verified regarding lack of degradation-related impurities by GC–MS.

2. Experimental

2.1. Instrumentation

The CE systems used were a CAPEL 105 (Lumex, St. Petersburg, Russia) and an Agilent HP^{3D}CE (Waldbronn, Germany). Separations were performed on fused-silica capillaries of 75 μm I.D. × 40 cm (30.5 cm effective length) or 48 cm (40 cm effective length), respectively. Ultrasonic irradiation was applied by a Transsonic 460/H sonifier (Elma, Singen, Germany) equipped with the sample vessel, in which the samples were immersed to be positioned into a water bath. A model HP6890 gas chromatograph (Agilent) was interfaced with a HP5973 mass detector. The gas chromatograph was equipped with a HP-5 column (30 m × 0.25 mm I.D.).

2.2. Reagents and solutions

High-purity water from a Millipore Synergy 185 UV ultrapure water purification system (Molsheim, France) was used for preparing the stock and buffer solutions, hydro-organic mixtures, and for conditioning the capillary. All chemicals employed in this research were of analytical-reagent grade. Acetone and 2-propanol were the products of Carl Roth (Karlsruhe, Germany). Pure solvents and their aqueous mixtures (50%, v/v) were used in sample preparation. The buffer solution consisted of 100 mM solutions of NaH_2PO_4 and Na_2HPO_4 (Merck, Darmstadt, Germany) mixed in appropriate volumes and finally adjusted to pH 7.4 with 0.1 M NaOH. The background electrolyte (BGE) was prepared by diluting the stock buffer solution with the deionized water and mixing with a 200 mM stock solution of sodium dodecyl sulfate (SDS; from Fluka, Buchs, Switzerland) and 2-propanol after appropriate dilutions. Tris(8-quinolinolato)gallium(III) (KP46; Fig. 1), tablet matrix, and formulated material were provided by the Faustus Translational Drug Development AG (Vienna, Austria). 8-Quinolinol (HQ) was purchased from Riedel-de Haën (Seelze, Germany) and purified by sublimation before use.

2.3. Sample preparation

The procedure comprised grinding the tablet into powder using an agate mortar and weighing 5 mg into a 10 ml flask. After adding 3 ml acetone–water (1:1), the flask was capped and placed into the ultrasonic bath. The active substance was extracted for 10 min at 25 °C, and the resultant suspension was filtered with a 0.45 μm membrane filter (Sartorius, Göttingen, Germany), 20-fold diluted with acetone–water (1:1) and analyzed by CE.

The artificial tablets were used for the optimization of the sample treatment and validating the proposed method. Their in-lab preparation was an easy process that simply entailed hand mixing of the tablet matrix with KP46, followed by addition of a given amount of HQ to mimic the drug-related impurity. The matrix constituted starch, lactose, polyvinylpyrrolidone, and magnesium stearate.

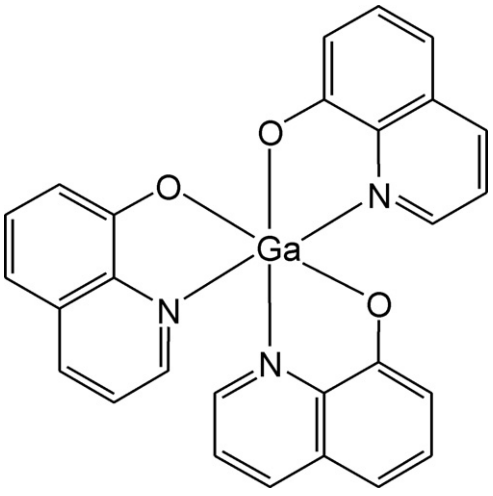

Fig. 1. . Structural formula of KP46.

2.4. Electrophoretic analysis

The BGE was a 50 mM SDS solution in 10 mM phosphate buffer (pH 7.4), containing 25% (v/v) 2-propanol, which was the optimum for separating KP46 from HQ and matrix components. Before use the BGE was passed through a 0.45 μm disposable filter and degassed in the ultrasonic bath. The samples were injected hydrodynamically at 20 mbar for a specified below time. The capillary voltage was set at 15 kV, the temperature at 25 °C, and monitoring wavelengths at 200, 210 and 235 nm (figures show detection traces recorded at 210 nm). In order to maintain the capillary under optimal conditions, its surface was regenerated after each run by sequential washing with 0.1 M NaOH (2 min) and water (1 min) followed by the BGE (5 min). In addition, the capillary was activated every day by rinsing with the same solutions in the same sequence for 10, 10, and 20 min, respectively.

2.5. Gas chromatographic analysis

The sample (20 mg) was placed into the stopped glass vial (3 ml volume), which was closed with the ground cover. After 15 min heating in a thermostat at 80 °C, the vial was pulled out, cooled and the cover was removed. Into the turned-over cover 0.5 ml chloroform was added and 1 μl of the chloroform solution was directly injected. The injector and interface temperature was kept at 250 and 280 °C, respectively. The column temperature was 80 °C during the injection and then the temperature was increased at 35 °C/min up to 295 °C. Helium was used as the carrier gas at the flow rate of 2.3 ml/min. The mass detector operating in the electron impact mode was kept at 280 °C.

3. Results and discussion

The optimization sequence followed consisted of two main steps: the first, focused on the best separation and detection, and the second, on the sample preparation for the target analytes. However, in view of insufficient aqueous solubility and stability of KP46 [12], the initial optimization experiments have been aimed at selection of a proper solvent for preparing and processing drug's standard solutions.

3.1. Solubility and stability testing

Several criteria must be satisfied by the solvent used: it must well solubilize the drug in a solid matrix, taking into account fairly high contents of active substance in tablet material (see below) but cause no (or insignificant) dissolution of the matrix components, and the conditions of the treatment must not induce degradation of the native drug. Of four solvent combinations tried, i.e., 2-propanol, acetone and their 1:1 mixtures with water, only individual and aqueous acetone were able to dissolve KP46 at a 1×10^{-3} M level. On the other hand, none of the solvent systems explored displays

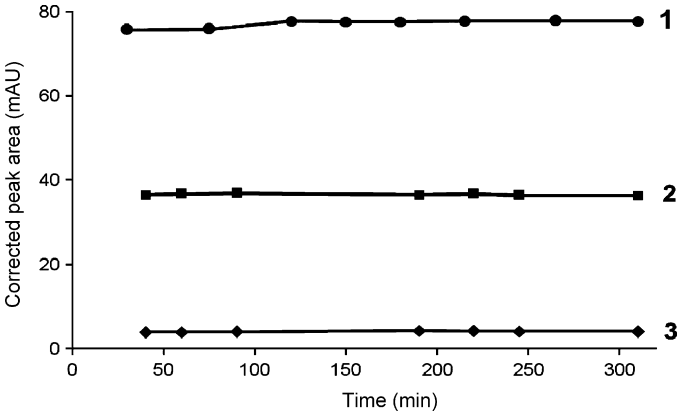

Fig. 2. Peak area vs. time of aging the KP46 solution in (1) water–2-propanol and (2) water–acetone (both 50%, v/v). Trace 3 refers to HQ from the water–acetone solution. The first kinetic point corresponds to the second CE run. Concentration: 1×10^{-4} M.

notable dissolving effect with regard to tablet matrix. Explicitly, the CE analysis of matrix leachates revealed that both 50% 2-propanol and 50% acetone extracts do not produce any peaks that could interfere with drug analysis. Still, acetone is preferential as causing negligible matrix effect. (Nonaqueous solutions were not subjected to these and the subsequent kinetic trials since injection of such sample plugs has repeatedly brought about voltage shutdown.)

Fig. 2 shows the peak area of KP46 dissolved in the respective hydro-organic medium as a function of time. From these profiles, it is evident that the parent complex retained its chemical integrity in either solution over a fairly long time. This appears not surprising given an extremely high thermodynamic stability ($\log \beta_n$ 40.7 [13]) and a moderate kinetic stability of KP46, the latter being commonly assessed in terms of the water exchange rate constant of a metal ion [14] (4.0×10^2 for Ga^{3+}). An uncommonly understood phenomenon in Fig. 2 (trace 3) is that the free ligand emerged immediately after the preparation of solution and its minor peak was seen in electropherograms from the beginning of CE kinetic measurements. Remarkably, the magnitude of the peak of HQ remained unchanged upon aging the KP46 solutions. This means that dissociation reaches a fast equilibrium and affects only a fraction of the intact complex.

In the following work, 50% aqueous acetone was adopted as sample preparation solvent with appropriate dissolving and polarity properties.

3.2. Optimization of the electrophoretic step

The optimization of the CE separation conditions was carried out with both standards and solutions from sample treatment, i.e. leachates from the artificial tablets (spiked with HQ) in order to take into account the presence of possible co-extracted matrix components which could exert an influence on the results (e.g., polyvinylpyrrolidone).

The influence of the SDS concentration in the BGE was first studied in an invariant way by maintaining the operational variables constant. The optimization was addressed to the resolution and shape of the analyte peaks that influences the detectability. As expected in micellar CE [15], increasing the concentration of SDS in the range 25–150 mM led to increased migration times of individual peaks accompanied by enlarged peak broadening. As a result, the resolution gradually decreased being affected for higher concentrations because of distorted peak shapes. In addition, the electric current was close to the maximum value (200 μA), when SDS increased to 150 mM. With the final 50 mM SDS concentration not only were resolution appropriate and short analysis time and a tolerable current combined, but also the signal response of KP46 was somewhat enhanced. However, even at such BGE conditions the peak shapes remained unsatisfactory. Similarly to our previously applied strategy [15], a further modification of the micellar electrolyte composition was directed toward incorporating an organic solvent as modifier of the aqueous phase [16]. The effect of varied concentrations of 2-propanol was explored and the best efficiency performance was achieved at 25%, as shown in Fig. 3a (acetonitrile and ethanol appeared to be less efficient for resolving the hydrophobic compounds in focus). One can evidence the principal advantage of using a micellar-mediated CE assay for the analysis of oral drug formulations, that is, the measurement of uncharged active substances in the presence of drug-related impurities, also bearing no charge.

3.3. Optimization of sample preparation

Sample treatment is required to be fast, providing complete recovery, and not altering the drug integrity. An ultrasound-assisted extraction meets these requirements for a wide range of solid matrices including pharmaceutical material. Furthermore, ultrasound energy does not only assist accelerated treatment of solid samples but also makes possible partial digestion of the tablets (no total dissolution of the sample was necessary). This method was tested in order to demonstrate its suitability for KP46 tablets without disturbing the target species. The solvent volume was found to be not

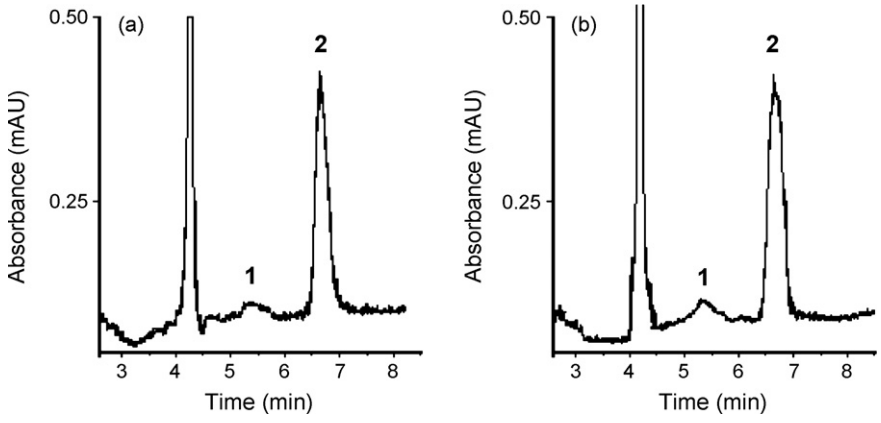

Fig. 3. Electropherograms obtained from (a) 1×10^{-4} M KP46 and (b) tablet analysis. Conditions: capillary, 48 cm (40 cm effective length) × 75 μm I.D.; electrolyte, 50 mM SDS, 10 mM phosphate buffer (pH 7.4), 25% (v/v) 2-propanol; temperature, 25 °C; sample introduction, 5 s at 20 mbar; detection at 210 nm. Peaks: (1) HQ; (2) KP46.

Table 1
Recovery of KP46 using different leaching times

Sonication time (min)	Corrected peak area (mAU)[a]		Recovery (%)[b]
	Leachate	Standard solution	
5	47.3 ± 0.6	47.3 ± 0.7	100.0 ± 0.5
10	47.5 ± 0.2	47.0 ± 0.9	101.3 ± 1.7
15	47.3 ± 0.4	47.4 ± 0.3	99.9 ± 1.5

[a] $n = 3$.
[b] Calculated by comparison of the peak area in a leached sample run to that obtained for a direct injection of 1×10^{-4} M standard solution of KP46.

an influential factor, and elevated temperatures were not needed. Therefore, the extraction time was only investigated with the ultrasonic bath. As can be seen in Table 1, the highest leaching efficiency was obtained starting from a 5 min sonication. However, a longer treatment (10 min) was selected for the extraction of tablet samples since performing pressurizing of tablet material might result in less yielding conditions.

3.4. Analytical characteristics

Linear regression analysis was performed using corrected peak areas at five levels of drug concentration in the range 2.5×10^{-5} to 1.5×10^{-4} M. The calibration graph was linear, included almost zero intercept, and the correlation coefficient (R^2) was 0.997. Intra-day and inter-day precision measurements were performed with a 1×10^{-4} M standard solution. The results indicate satisfactory repeatability (in terms of relative standard deviation; RSD) for migration time: 1.7% ($n = 6$). The repeatability RSD for corrected peak area was favorably below 1.0%. The value of peak area reproducibility assessed over three days was 0.7% RSD, thus demonstrating an excellent long-term stability of the method. While the CE procedure under development was aimed at rather high contents of KP46, the detection limit (LOD) was also obtained with three-time S/N ratio. The S/N was calculated from the peak height and the noise taken as the SD of the baseline (which was considered to be an average of 20 noise peaks near the analyte peak). For the proposed method, the LOD is 6×10^{-6} M.

3.5. Analysis of tablets

With the selected extraction conditions, real tablet samples were analyzed and an example of electropherogram is shown in Fig. 3b. There is no interference with the determination of KP46 whose peak migrates past the detection window well after the HQ peak, and the neighboring sample-matrix signals are absent. This allowed us to use the external calibration to accurately quantify the drug level in real samples. Because of very high concentrations of the concerned analyte, a dilution factor of 20 was adopted to match with the working range of the CE procedure. In order to avoid any uncertainty associated with possible analyte decomposition, a 1×10^{-4} M solution of KP46 was analyzed after each sample and used as external standard. The analysis results are summarized in Table 2. The recovery values range from 97 to 102% and their proximity to 100% can be taken as a sign of tablet stability (over 2 years of storage).

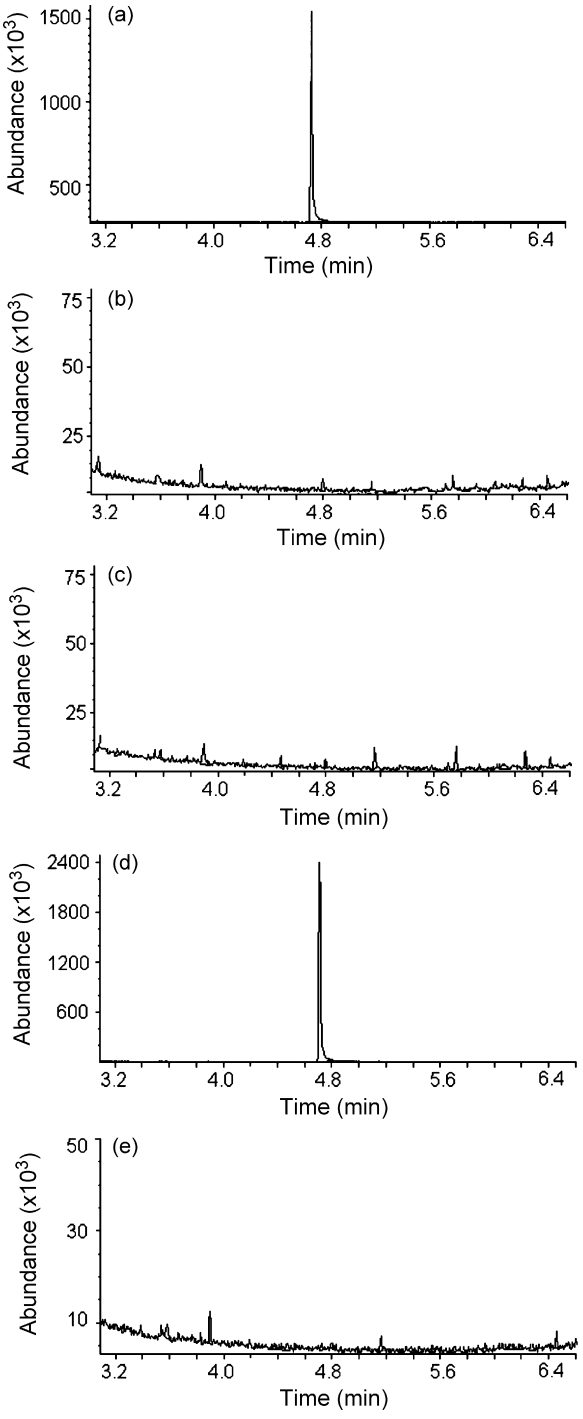

Fig. 4. GC–MS chromatograms corresponding to (a) HQ; (b) KP46; (c) tablet matrix; (d) an artificial tablet sample spiked with HQ (1%, w/w); and (e) tablet sample (30% KP46). Conditions as described in Section 2.5.

Table 2
Results of sample analysis by CE

Certified value (%)	Concentration of KP46 (M)[a]	Found, mean ± SD × 10^{-5} (M)[b]	RSD (%)	Recovery (%)
30	1×10^{-4}	9.67 ± 0.25	2.59	96.7
25	1×10^{-4}	1.01 ± 0.11	1.09	100.5
20	1×10^{-4}	1.02 ± 0.33	3.24	101.8
10	1×10^{-4}	9.97 ± 0.17	1.71	99.7

[a] In the sample after pre-treatment.
[b] Three aliquot portions were taken from each tablet and analyzed in triplicate.

The possibility that the ligand may exist in tablet formulation as an impurity or a degradation product at a concentration level below CE method's detectability was eliminated by performing a complementary GC–MS analysis. The method that enabled sensitive and selective detection of volatile analytes has been chosen due to differences in volatility between KP46 and HQ. Indeed, according to the data of thermogravimetric analysis, mass loss of HQ becomes

evident yet below the melting point (76 °C) [17], whereas KP46 displays no measurable volatility up to ≥150 °C [18]. Fig. 4 depicts a series of chromatograms obtained for the ligand sample, an artificial tablet amended with HQ to yield a concentration of 1%, and the real tablet (30% KP46) (the GC–MS profiles of KP46 and tablet matrix are also included for illustration). As anticipated, only the former two encompassed the MS signal of HQ. The results provided by the GC–MS method thus unequivocally indicated that the tablets contain no ligand impurity (of any possible origin).

4. Conclusions

This study demonstrates a useful CE method developed to afford reliable determination of a gallium-based anticancer drug in solid dosage form. Preparation of samples for this method is quite simple. Furthermore, the effect of the tablet matrix is negligible giving accurate quantifications as proved by good agreement with labeled amounts for the active substance. On the basis of CE results for several lots of tablets and supplementary GC–MS analysis, it was concluded that the tablet samples were stable and not degraded within a two-year period. The same analytical strategy can be recommended for a similar assessment of other metal-containing pharmaceuticals, as well as for profiling degradation-related impurities, provided that the analyte contents in formulations are moderately high.

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