Journal of Bioanalysis & Biomedicine - Open Access
JBABM/Vol.2 Issue 2

Research Article | OPEN ACCESS Freely available online | doi:10.4172/1948-593X.1000018

Pharmacokinetics of Casiopeína IIgly in Beagle Dog: A Copper Based Compound with Antineoplastic Activity

Cañas-Alonso RC[1], Fuentes-Noriega I[1] and Ruiz-Azuara L[2]*

[1]Facultad de Química, Departamento de Farmacia, Universidad Nacional Autónoma de México, 04510 México D.F., México
[2]Facultad de Química, Departamento de Química Inorgánica y Nuclear, Universidad Nacional Autónoma de México, 04510 México D.F., México

Abstract

Casiopeína IIgly is a mixed chelate coordination complex with copper core that have demonstrated high antineoplastic activity *in vitro* and *in vivo*. In the present work, a developed and validated method for measurement of Casiopeína IIgly in beagle dog and its application in a pharmacokinetic study is reported. The analyte was isolated from blood by solid-phase extraction using Strata X cartridges. The analysis was carried out on a Synergy Polar-RP column (30 X 2.0 mm) using an isocratic elution and MeOH/HFBA 0.1% (4:6) as the mobile phase. An Agilent LC/MSD Trap VL equipped with an ionization electrospray source, was operated in selective ion storage (SIS) using stable ion $[Cu^{(II)}(F_7C_3COO)$ 4,7-dimethyl phen $]^+$ with 484 m/z for quantification result of ESI reaction between Casiopeína IIgly and HFBA. The method demonstrated to be linear (r = 0.9992) in the range from the 0.1 to 15 µg/ml with a limit of detection (LOD) of 50 ng/ml. All the parameters of validation such as selectivity, accuracy, precision and recovery were within the required limits. Pharmacokinetics assay was carried out at 2 doses, indicated a high elimination rate.

Keywords: Pharmacokinetics; Copper; HPLC-MS; Metal-based compound; Casiopeína IIgly

Introduction

At the present time, metal based drugs are an important source of novel molecules with therapeutic activity. Particularly, coordination compounds with copper core have been investigated in the last few decades after the discovery of cisplatin, because they have shown good antineoplastic activity and less toxicity, being copper an essential element (Wang et al., 2006; Marzano et al., 2009).

Casiopeínas® are a "the novo" group of coordination compounds with copper that have demonstrated high antineoplastic activity *in vivo* and *in vitro* (Ruiz-Ramírez et al., 1991; Gracia et al., 2001; Carvallo et al., 2008; Trejo et al., 2005; Mejia and Ruiz-Azuara, 2008). These compounds have a general formulae $[Cu(N\text{-}N)(N\text{-}O)H_2O]NO_3$ (where N-N = diimines as 1,10-phenanthroline, 2,2-bypiridine, or substituted and N-O = aminoacidate) or $[Cu(N\text{-}N)(O\text{-}O)H_2O]NO_3$ (where N-N = diimines as 1,10- phenanthroline, 2,2-bypiridine, or substituted and O-O = acetylacetonate, salycilaldehidato) and have been patented (Ruiz-Azuara, 1996; Ruiz-Azuara, 1997). Some stability constant and structural data are published (Gasque et al., 1992; Ruiz-Ramírez et al., 1992; Ruiz-Ramírez et al., 1993; Álvarez et al., 1995) and QSAR studies have been reported for this group of mixed chelate complexes (Bravo-Gómez et al., 2009).

Casiopeína IIgly ((4,7-dimethyl-1,10-phenanthroline) (glycinato) copper (II) nitrate) (Ruiz-Ramírez et al., 1993) (Figure 1) has shown a good citotoxic activity and low toxicity in comparison with the others Casiopeínas compounds. This potentially useful antineoplastic agent is active against murine and human cancer cell lines, including those resistant to other drugs (De Vizcaya-Ruiz et al., 2000). Its mechanism of action has not been fully defined yet, but it has been reported that Casiopeína IIgly uncoupled the cell respiratory chain by interaction with succinate and 2-oxoglutarato dehydrogenases (Marín-Hernández et al., 2003), increased reactive oxygen species (ROS) (Alemón-Medina et al., 2007; Alemón-Medina et al., 2008), induces apoptosis in glioma C6 through caspase-dependent and caspase-independent mechanisms depending of doses used (Trejo-Solís et al., 2005; Mejia and Ruiz-Azuara, 2008) and binds to DNA with high affinity (Rivero-Müller et al., 2007). Toxicity studies (De Vizcaya-Ruiz et al., 2003; Hernández Esquivel et al., 2006; Leal-García et al., 2007) have been reported and are still in progress. Also, Casiopeína II gly has shown antitumor activity on HCT-15 carcinoma in vitro and in a nude mice assay and induction of apoptosis have been observed (Carvallo et al., 2008). Besides has shown activity in Hela, SiHa, MCF7 cellular lines *in vitro* (Bravo-Gómez et al., 2009).

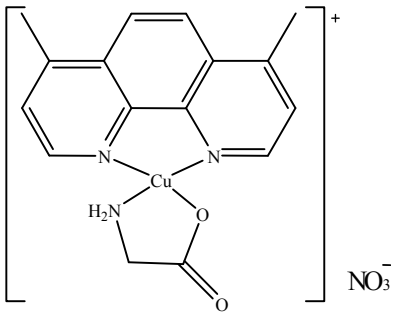

Figure1: Casiopeína IIgly chemical structure.

***Corresponding author:** Lena Ruiz-Azuara, Ph.D. Facultad de Química. Departamento de Química Inorgánica y Nuclear, Universidad Nacional Autónoma de México, 04510, Tel/Fax: (5255) 56223529; E-mail: ruizazuara@gmail.com

Received January 27, 2010; **Accepted** March 22, 2010; **Published** March 22, 2010

Citation: Cañas-Alonso RC, Fuentes-Noriega I, Ruiz-Azuara L (2010) Pharmacokinetics of Casiopeína IIgly in Beagle Dog: A Copper Based Compound with Antineoplastic Activity. J Bioanal Biomed 2: 028-034. doi:10.4172/1948-593X.1000018

Few methods have been developed to quantify Casiopeínas in biological fluids and all of them were based in UV detection (Fuentes-Noriega et al., 2002; Reyes et al., 2003). An HPLC-UV method to determinate Casiopeína IIgly in rat plasma over the range 2.5 to 50 µg/ml have been reported and was used for some assessments. Nevertheless, new toxicological data and related assays have demonstrated the necessity of quantify this coordination complexes in lowest blood concentrations, therefore more sensitivity is required. On the other hand, the previously reported methods have excessive sample volume requirements for application to preclinical pharmacokinetics studies. In this communication, the authors report the first method for measurement of this coordination complex using MS detection and smaller sample volume.

In order to continue with the pharmacokinetics investigation, the aim of this research work was to develop and validate a sensitive, specific, robust and rapid ion pair reversed phase LC-MS method of Casiopeína IIgly measurement in total blood, using ion 484 m/z result of an interphase reaction for quantification. Ketoprofen was used as internal standard. Analyte was isolated by solid-phase extraction using Strata X cartridges. The method was successfully applied in a pharmacokinetics study in beagle dog.

Experimental

Chemical and reagents

Casiopeína IIgly ([Cu(4,7-dimethyl,1,10-phenathroline) (glycinate) H2O] NO3·H2O was provided by the Department of Inorganic and Nuclear Chemistry (School of Chemistry, UNAM) and was synthesized following the procedure reported in previous patents (Ruiz-Azuara, 1996; Ruiz-Azuara, 1997). Ketoprofen was acquired from Kendrick Laboratories (Mexico). Heptafluorobutyric acid (HFBA), ammonium formate, ammonium acetate, aluminum chloride and formic acid was purchased from Sigma-Aldrich. Ammonium sulfate was obtained from Merck. Methanol and acetonitrile (both chromatographic grade) and zinc sulfate from J.T. Baker and the deionized water was generated in-house using a Milli-Q system from Millipore (MA. USA).

Liquid chromatographic/ mass spectrometer conditions

Liquid chromatography separation and mass spectrometry detection were performed using an Agilent LC (Waldbronn, Germany)-MS (Palo Alto CA, USA) system. This equipment consists in an 1100 quaternary pump (G1311A), an 1100 autosampler (model G1313A) and a Mass Trap VL spectrometer equipped with an electrospray ionization source. The software used for data processing was LC-MSD Trap version 5.2.

The selected column to achieve separation was a Synergy Polar RP (4µm, 30 mm X 2 mm, 4µm particle size, Phenomenex) preceded by a security guard cartridge (4 X 3 mm, Phenomenex) and the mobile phase used MeOH/ HFBA 0.1 % (4:6). The flow was carried out as follow: 0.5 ml/min in the first stage (0-4.5 min) and the rest of chromatographic run until 10 min was 0.9 ml/min.

Mass spectrometer conditions were divided in two segments: values of nebulizer, dry gas, capillary, capillary exit offset and trap drive for Casiopeína IIgly quantification (from 0 to 4.5 min) were 40 psi, 8 L/min, -4500 V, 61.48V and 47.4 and for Ketoprofen (I.S.) (from 4.5 to 10 min) were 50 psi, 10 L/min, -2385V, 50 V and 49, respectively. Target, maximum accumulation, time and average were 200 000, 50 ms and 17 for both segments. Mass spectrometer was operated under positive ion mode and nitrogen was used as drying gas at 350°C. Selective ion storage for stable ions [$Cu^{(II)}$ (F_7C_3COO) 4,7-dimethyl phen]$^+$ with 484 m/z for Casiopeína IIgly and ketoprofen adduct with 299 m/z ([M-H+2Na]$^+$) as internal standard was used for quantification. These fractions were confirmed through MS^n experiments.

Preparation of samples

Samples were prepared dissolving an accurate weight of Casiopeína IIgly in deionized water to obtain concentration of 1 mg/ml at room temperature. Working standards of Casiopeína IIgly dissolved in water in a concentration range of 10 and 1000 µg/ml were prepared by independent dilution. Calibration curve with the concentrations of 0.1, 0.5, 1, 10 and 15 µg/ml and quality control samples (0.3, 5 and 12 µg/ml) were obtained by spiking the appropriate solution to 2 ml of dog blood. I.S. dissolved in MeOH/ HFBA 0.1 % (4:6) at concentration of 25µg/ml was prepared from 1 µg/ml of Ketoprofen in methanol stock solution.

Sample treatment

For the protein precipitation, methanol, acetonitrile and solution as aqueous aluminum chloride (10% w/v), aqueous ammonium sulfate (saturated at room temperature), zinc sulfate heptahydrate (10% w/v) were mixed of MeOH/HFBA and Acetonitrile/HFBA in different proportions and assayed.

A satisfactory treatment was found when 0.5 ml aliquot of beagle dog blood was diluted with 600 µL of HFBA 5% in water (v/v) followed by 300 µL of acetonitrile. The mixture was vortexed for 30 seconds after each addition and centrifuged for 5 minutes at 15000 rpm.

Extraction

The cartridges Strata X (33µm, 60 mg, 1 cc, Phenomenex) were prepared passing first 0.5 ml of methanol twice and later 0.5 ml of HFBA to 5% with a Waters manifold system (Milford, MA, USA). The mixture was added to the cartridges and washed with 300 µL of HFBA to 5%. Cartridges were dried for 3 minutes and eluted with 0.7 ml of dichloromethane. Samples were concentrated to dryness at 30°C under nitrogen. The residue was reconstituted in 100 µL of I.S. solution followed by vigorous stirring until dissolution. A 1 µL of mixture was injected into HPLC/MS system for analysis.

HPLC-MS validation

Specificity and Matrix effect

To investigate possible interference by endogenous compounds, various lots of blood from different beagle dogs were extracted and analyzed. Matrix effect was determined according with Matuszewski methodology (Matuszewski et al., 2003). Blanks from 6 lots of beagle dog blood were processed following extraction steps. Before evaporation of dichloromethane, adequate concentration (0.3, 5 and 12 µg/ml, n=3) of Casiopeína IIgly dissolved in mobile phase was added and injected to HPLC-MS

system. Matrix effects were determined by comparing LC-MS response of a blank of blood reconstituted with Casiopeína IIgly and LC-MS response of analyte in mobile phase at same concentration.

Linearity

To test linearity, five calibration curves of spiked samples with Casiopeína IIgly were evaluated. Calibration curves were represented by plotting the peak area ration of Casiopeína IIgly/ ketoprofen (IS) versus the concentrations of standard curve. The linearity was tested using different weighted linear regression of the data.

Limit of quantification and limit of detection

The Limit of Quantification (LOQ) was defined as lowest concentration on the standard line that could be measurement with accuracy and precision according with to FDA guidance recommendations. For LOQ experiments, 0.1 µg/ml concentration was evaluated through 5 days of validation. On the other hand, Limited of Detection (LOD) was defined as the sample concentration resulting in a peak area of three times the noise level according with the following mathematical expression: LOD = (3SDB/ m). Standard deviation of the blank (SDB) was calculated using 6 blanks and the slope (m) was obtained from calibration curve of Casiopeínas IIgly.

Precision and accuracy

The precision (intra- and inter-day) and accuracy of the validation was assessed from quality control samples on 3 levels (0.3, 5 y 12 µg/ml) per quintuplicate through 5 validation days. Each day of validation a fresh calibration curve with 5 levels was realized and processed samples were interpolated to obtain experimental concentration.

Recovery

For recovery assay, done by triplicate, blood samples were spiked with three different quantities (0.3, 5 and 12 µg/ml) of Casiopeína IIgly. Samples were analyzed as described in the section of sample preparation. The recovery was determined by comparing the relative peak of extracted samples from blood

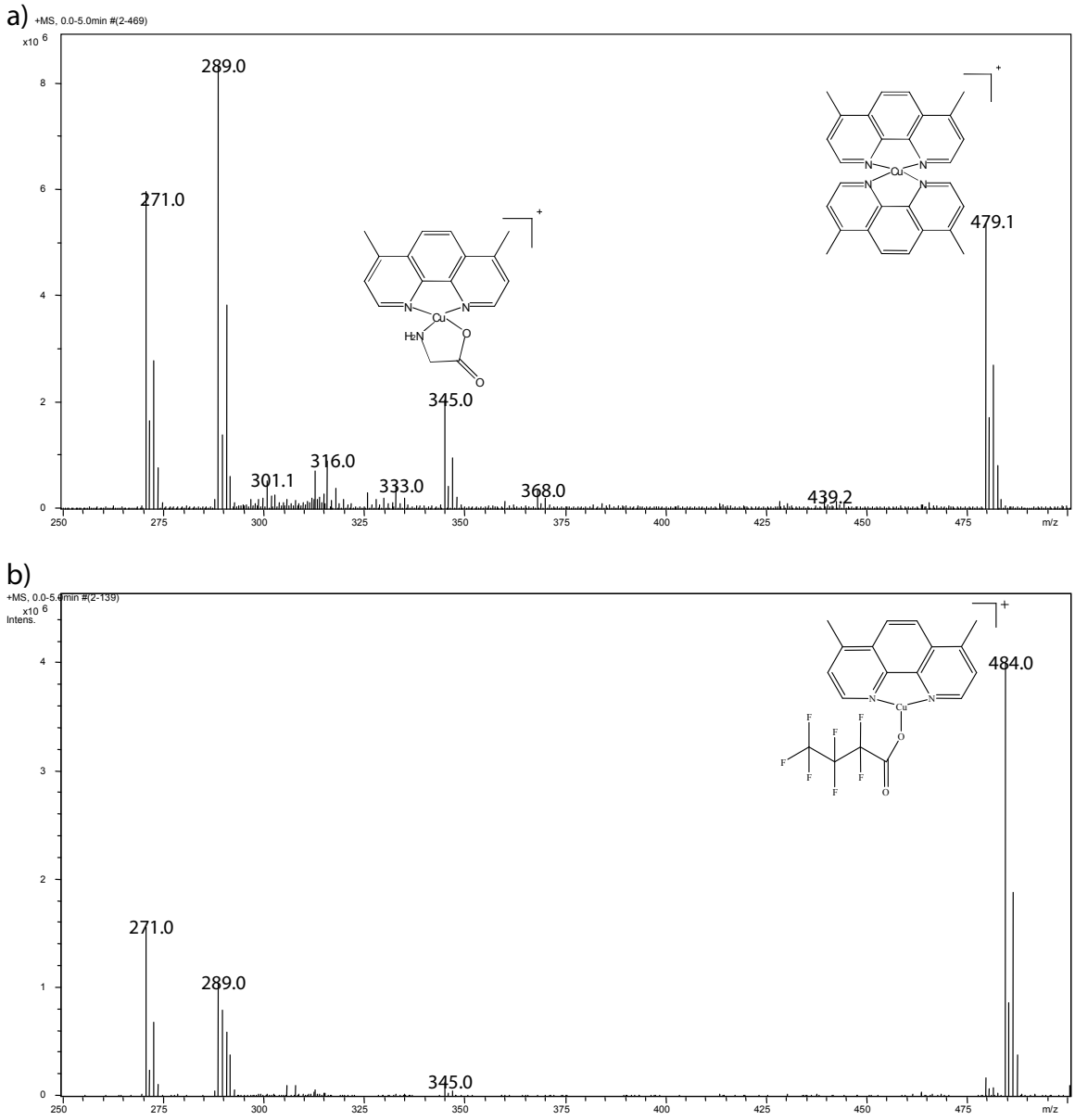

Figure 2: Mass spectrum of Casiopeína IIgly dissolved in a) Methanol and b) MeOH/HFBA 0.1% (4:6). 484 m/z ($[Cu^{(II)}(F_7C_3COO)$ 4,7-dimethyl phen $]^+$) ion is the result of Casiopeína IIgly and HFBA ESI reaction.

versus the relative peak of blanks spiked post-extraction with Casiopeína IIgly at the same level.

Stability

Free-thaw stability was carried out per triplicate of quality control samples (0.3, 5 and 12 µg/ml) at 24, 48, 72 hours, 7 and 15 days. At defined day, stored samples at -20°C were thawed at room temperature and an aliquot of 500 µL was taken and analyzed.

In order to evaluate possible sample degradation stored in autoinjector, spiked samples of Casiopeína IIgly at 3 leves of QC (n=3) were extracted and reconstituted, according to the development method. Samples were injected over a period of 24 hours in intervals of 3 hours. Assay for short term stability was determined at 3 levels of QC by triplicate. Samples were prepared and kept at room temperature, for a period that exceed the preparation time of the samples (around 8 hours).

Preclinical pharmacokinetics assay

Pharmacokinetic assay was performed under approval of the Internal Committee Postgraduate of the Veterinarian School at National Autonomous University of Mexico, Mexico City, Mexico. Four male beagle dogs weighting 14.4 ± 2.86 Kg were administered a continuous intravenous infusion (60 minutes) of Casiopeína IIgly in a solution of 5% glucose at doses of 3 mg/Kg and 1.5 mg/Kg (two beagle dog for each dose) after an overnight fasting. Blood samples (1 ml) taken from the cephalic vain were collected into heparinized tubes at 20, 40, 60, 80, 120 and 160 minutes from the beginning of infusion and stored at -20°C prior to analysis.

Results and Discussion

Analytical method development

Initial retention experiments on C_8, C_{12}, and C_{18} column (Synergy Hydro RP (C_{18}, 150mm X 4.6 mm, 4µm), Synergy Max RP (C_{12}, 150mm X 4.6 mm, 4µm), Zorbax Eclipse XDB (C_8, 150mm X 4.6 mm, 4µm) and Agilent Eclipse (C_{18}, 150mm X 4.6 mm, 5µm) and various mobile phases of different compositions (aqueous ammonium formate, aqueous ammonium acetate, formic acid) with various organic phase ratios (15:85-90:10) demonstrated that Casiopeína IIgy is unstable in standard chromatographic conditions; dissociation of the coordination complex

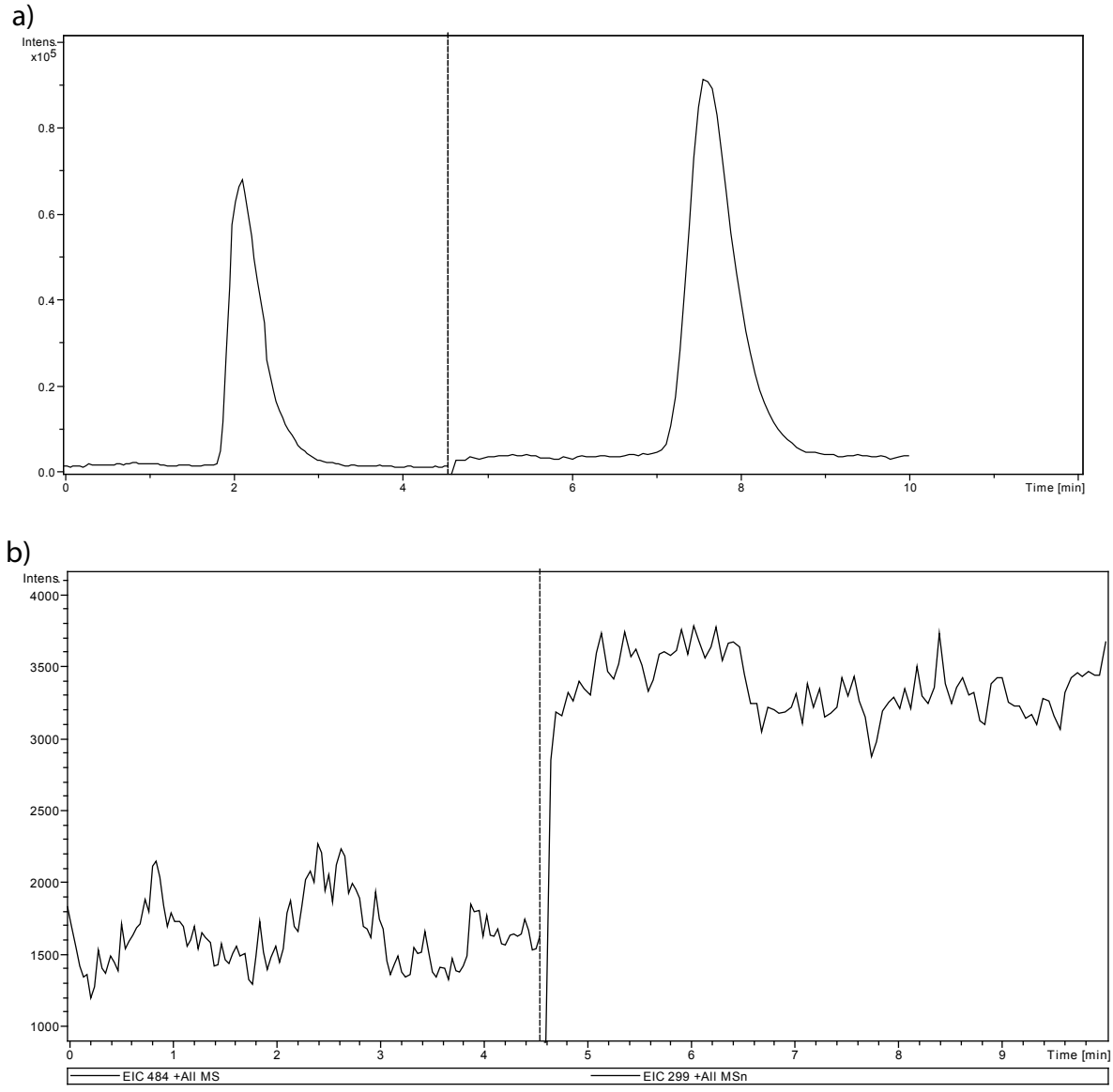

Figure 3: Chromatograms obtained from a) blood sample spiked with 1 µg/mL of Casiopeína IIgly (tr= 2.1) and ketoprofen (tr = 7.7) as IS and b) blank blood.

was found in every assay, due to rupture of bonds between bidentade ligand glycinate and copper. Although ternary copper complexes such as $[Cu^{(II)}(RCOO)phen]^+$ are known to have large stability constant in solution ($\beta > 10^{16}$) (Yamauchi et al., 1985), the assaying showed that this compound can be un-coordinated by the effect of partition chromatography.

According to these results and in order to obtain retention as well as good resolution and a symmetric peak shape in the reverse phase, a methodology has been developed on the base of columns with aromatic stationary phase and an ionic pairing agent. π-interaction between stationary phase and 4,7-dimethyl,1,10-phenathroline which is part of the Casiopeína IIgly molecule could occur in order to stabilize the complex. Among the columns tested, Synergy polar RP (150mm X 4.6 mm, 4µm) was chosen because of its good resolution and peak shape. Mobile phases containing methanol were selected instead of others as they help to increase the π interaction between aromatic systems. Ion-pair agent (HFBA) was an option to develop the analytical method because Casiopeína IIgly is a highly soluble coordination compound in polar solvents. Furthermore, the formed ion $[Cu^{(II)}(F_7C_3COO)$ 4,7-dimethyl phen $]^+$ was stable during electrospray and displayed a much greater intensity than others (Figure 2). The best proportion of mobile phase was Methanol/HFBA 0.1% in water (4:6 v/v) and was found varying the concentration of ion-pairing (from 0.1 to 5%) and correlating with retention time and peak shape.

Method validation

According to method developed for quantification of this coordination complex, the retention times for Casiopeína IIgly and Ketoprofen in segmental optimal conditions were 2.1 min and 7.7 min respectively (Figure 3).

The parameters of full validation assayed in this work were matrix effects, linearity, accuracy, precision, sensitivity, recovery and stability and was conducted conforming to currently accepted U.S. Food and drug Administration (FDA) bioanalytical method validation guide (FDA Guidance 2001).

All samples were found to be free of interferences with the compound of interest. All ratios of the peak area resolved in the blank sample compared with resolved by the mobile phase, are between 85 and 115%, which means no significant matrix effects at 3 levels determinate.

Linear relationship was found without weighting factors in the range of concentrations from 0.1 to 15 µg/ml. The simple linear equation that describes the behaviour was y = -0.216+0.873X and correlation coefficient mean of calibration curves was 0.9992 ± 2.9 E-04. For Limit of Quantification (LOQ) assay, the lowest concentration of standard curve was evaluated on five validation days and average values of 13% and 107 % of precision and accuracy was found, respectively. These data are in agreement with FDA recommendations, consequently 0.1 µg/ml was considered as LOQ. Limit of detection (LOD) was obtained through mathematical expression previously described in HPLC-MS validation section and the result indicated a value approximately of 50 ng/ml.

The precision and accuracy of the method were evaluated using spiked plasma at concentrations of 0.3, 5 and 12 µg/ml As shown in Table 1, the maximum coefficients of variation for intra-day and inter-day assay were 7.7 % and 11.6 % respectively. The accuracy was between 109% and 97% of the nominal value at the three levels. These results indicate that the method is reproducible and accurate.

The extraction recovery determined for Casiopeína IIgly at three levels of Quality controls (n=3) were shown to be consistent, precise and repeatable. A satisfactory recovery of 65% was obtained.

The stability of Casiopeína IIgly was investigated under three conditions: short term stability, post-treatment stability (stored on injector) and freeze and thaw stability. Table 2 summarized these data. Results demonstrated that Casiopeína IIgly is stable in blood at least 15 days and there were no stability related problems during the routine analysis of samples for pharmacokinetics study.

Application of analytical method in pharmacokinetic study

The fully validated HPLC-IT-MS method was used in a pharmacokinetic assay of Casiopeína IIgly in blood dog at 2 doses (1.5 and 3 mg/kg, n=2) and the study was designed with the aim of characterizer the steady state part. The pharmacokinetics data were tested by compartmental and no compartmental models, using WINNONLIN software (4.0.1 version). According to static parameters, no compartmental model was chosen. The relevant pharmacokinetics parameters as $t_{½}$, clearance (Cl), Volume of distribution (Vd) and Area Under the Curve (AUC_{0-})

QC sample	Concentration (µg/ml)	Mean measured concentration (µg/ml)±S.D.	Precision C.V.(%)	Accuracy(%)
Intra-day				
LQC	0.3	0.328±0.025	7.62	109.33
MQC	5	4.732±0.139	1.17	98.92
HQC	12	11.871±0.139	1.17	98.92
Intra-day				
LQC	0.3	0.317±0.037	11.64	105.66
MQC	5	4.845±0.194	4.00	96.95
HQC	12	11.833±0.340	2.87	98.62

Table 1: Intra-day and inter-day accuracy and precision of Casiopeína IIgly at 3 concentration levels (n=5).

Concentration (µg/ml)	Accuracy (mean ± S.D.)%		
	Short term stability	Freeze and thaw stability	Postpreparative stability
0.3	101.9 ± 6.3	104.2 ± 5.8	95.4 ± 7.9
5	95.6 ± 4.2	96.0 ± 4.7	98.8 ± 4.2
12	97.5 ± 6.8	98.2 ± 6.5	97.5 ± 6.8

Table 2: Data showing stability of Casiopeína IIgly in beagle dog blood at 3 levels of QC.

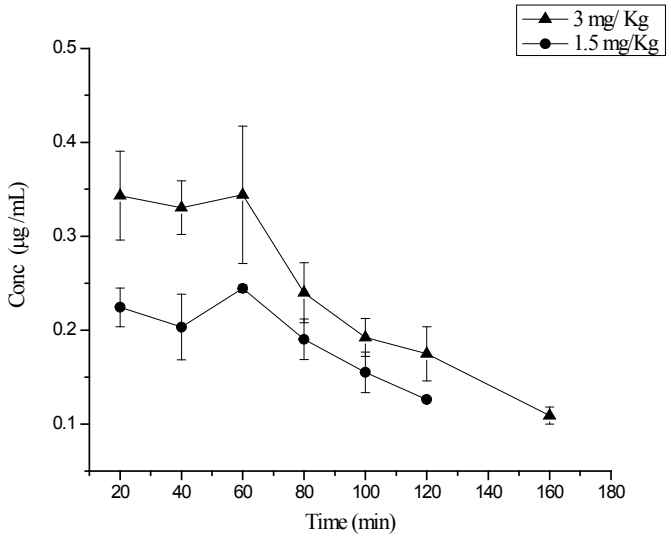

Figure 4: Blood concentration vs time profile of Casiopeína IIgly during and after i. v. infusion administration in beagle dog. Two doses (1.5 and 3.0 mg/Kg) were assayed.

for doses of 1.5 mg/Kg are as follow: 2.45± 0.04 h, 1460.25±170.9 mL/h kg, 5153.25 ± 89.3 mL/Kg and 0.345 ± 0.04 µg h/mL and for 3 mg/Kg were 1.905 ± 0.45 h, 3090.55±669.67 mL/h kg, 8267.17 ± 268.61 mL/Kg and 0.512 ± 0.9 µg h/mL respectively.

In this work, the steady state levels in beagle dog blood obtained following continuous infusion of Casiopeína II gly in both doses were achieved rapidly (Figure 4). There are few pharmacokinetics data reported for this coordination complex but the results as the elimination half–life observed in beagle dog was similar to previously reported in rats assay.

In conclusion, the HPLC-MS method developed and validated for quantification of Casiopeína IIgly in beagle dog blood demonstrated to have good precision, accuracy acceptable recovery, low limits of quantification and detection and can be used for toxicokinetics and related assays. For beagle dog the pharmacokinetics parameters such as clearance and half life were obtained and demonstrated a high elimination rate. These results will be useful tool in the pharmacokinetics study of Casiopeína IIgly in clinical trials.

Acknowledgements

This work was financially supported by PAPIIT IN204706 and CONACYT 60085, and Sectorial Salud C01-2002-7677. The authors thank Ma. Elena Bravo for Casiopeína preparation and Manuel Fuentes and Héctor Rico for technical assistance in animal handling. One of us (Cañas-Alonso) thanks for CONACyT for Doctoral scholarship.

References

1. Alemón-Medina R, Breña-Valle M, Muñoz-Sánchez JL, Ruiz-Azuara L, Gracia-Mora I (2007) Induction of oxidative damage by Copper-based antineoplastic drug (Casiopeínas). Cancer Chemother Pharmacol 2: 219-228. » CrossRef » PubMed » Google Scholar

2. Alemón-Medina R, Muñoz-Sánchez JL, Ruiz-Azuara L, Gracia-Mora I (2008) Casiopeína IIgly induced cytotoxicity to HeLa cells depletes the levels of reduced glutathione and is prevented by dimethyl sulfoxide. Toxicol In Vitro 22: 710-715. » CrossRef » PubMed » Google Scholar

3. Álvarez A, Briansó JL, Piniella JF, Moreno-Esparza R, Ruiz-Ramírez L, et al. (1995) Crystal Structure of di-chloro-bis nitrato-(4, 4´-dimethyl-2,2´bypiridine (copper(II)), [CuCl(NO_3)($C_{12}H_{12}N_2$)]. Zeitschrift für Kristallographie 210: 543-544. » CrossRef » PubMed » Google Scholar

4. Bravo-Gómez ME, García-Ramos JC, Gracia-Mora I (2009) Antiproliferative activity and QSAR study of copper(II) mixed chelate [Cu(N–N)(acetylacetonato)]NO_3 and [Cu(N–N)(glycinato)]NO_3 complexes, (Casiopeínas®). J Inorg Biochem 103: 299-309. » CrossRef » PubMed » Google Scholar

5. Carvallo-Chaigneau F (2008) Efectos antiproliferativos y apoptoticos de las casiopeinas(R) IIgli y III-ia en lineas tumorales humanas. Ph D. dissertation, National Autonomous University of Mexico, Mexico. » CrossRef » PubMed » Google Scholar

6. De Vizcaya-Ruiz A, Rivero-Müller A, Ruiz-Ramírez L, Howarth JA, Dobrota M (2003) Hematotoxicity response in rats by the novel copper-based anticancer agent: Casiopeína II. Toxicology 194: 103-113. » CrossRef » PubMed » Google Scholar

7. De Vizcaya-Ruiz A, Rivero Muller A, Ruiz-Ramírez L, Kass GEN, Kelland LR, et al. (2000) Induction of Apoptosis by Novel Copper-based Anticancer Compund-Casiopeína II in L1210 Murine Leukaemia and CH1 Human Ovarian Carcinoma Cells. Toxicol in vitro 14: 1-5. » CrossRef » PubMed » Google Scholar

8. FDA (2001) US Department of Health and Human Service, Food and Drug Administration, Center for Drug Evaluation and Research (CDER). » CrossRef » PubMed » Google Scholar

9. Fuentes-Noriega I, Ruiz-Ramírez L, Tovar A, Rico-Morales H, Gracia-Mora I (2002) Development and Validation of a liquid chromatographic method for Casiopeína III-i in rat plasma. J Chromatogr B 772: 115-121. » CrossRef » PubMed » Google Scholar

10. Gasque L, Ruiz-Ramírez L, Moreno-Esparza R (1992) Stabilities of ternary copper and nickel complexes with 1,10 phenanthroline. J Inorg Biochem 48: 121-127. » CrossRef » PubMed » Google Scholar

11. Gracia I, Ruiz-Ramírez L, Romero L, Márquez A, Gómez-Ruiz C, et al. (2001) Knight´s Move in the Periodic Table, from Copper to Platinum, Novel antitumor mixed chelate copper compounds, Casiopeínas, Evaluated by *In vitro* Human and murine cancer cell line panel. Metal Based Drugs 8: 19-28. » CrossRef » PubMed » Google Scholar

12. Hernández-Esquivel L, Marín-Hernández A, Pavón N, Carvajal K, Moreno-Sánchez R (2006) Cardiotoxicity of copper-based antineoplastic drugs casiopeinas is related to inhibition of energy metabolism. Toxicol Appl Pharmacol 212: 79-88. » CrossRef » PubMed » Google Scholar

13. Leal-García M, García-Otuño L, Ruiz-Azuara L, Gracia-Mora I, Luna-del Villar J, et al. (2007) Assessment of Acute Respiratory and Cardiovascular Toxicity of Casiopeínas in Anaesthetized Dogs. Basic Clin Pharmacol Toxicol 101: 151-158. » CrossRef » PubMed » Google Scholar

14. Marín-Hernández A, Gracia-Mora I, Ruiz-Ramírez L, Moreno-Sánchez R (2003) Toxic effects of copper-based antineoplastic drug (Casiopeínas) on mitochondrial functions. Biochem Pharmacol 65: 1979-1989. » CrossRef » PubMed » Google Scholar

15. Marzano C, Pellei M, Tisato F, Santini C (2009) Copper complexes as Anticancer Agent. Anticancer Agent Med Chem 9: 185-211. » CrossRef » PubMed » Google Scholar

16. Matuszewski BK, Constanzer ML, Chavez-Eng CM (2003) Strategies for the Assessment of Matrix effect in Quantitative Bioanalytical Methods Based on HPLC-MS/MS. Anal Chem 75: 3019-3030. » CrossRef » PubMed » Google Scholar

17. Mejia C, Ruiz-Azuara L (2008) Casiopeinas IIgly and IIIia induce Apoptosis in Medulloblastoma Cells. Phatol Oncol Res 14: 467-472. » CrossRef » PubMed » Google Scholar

18. Rivero-Müller A, De Vizcaya-Ruiz A, Planc N, Ruiz L, Dobrota M (2007) Mixed Chelate Copper Complexes, Casiopeína IIgly, Binds and Degrades nucleic acids: A Mechanism of Cytotoxicity. Chem Biol Interact 165: 189-199. » CrossRef » PubMed » Google Scholar

19. Ruiz-Azuara L (1996) Process to obtain new mixed cooper amino-acidate from methyl phenanthroline complexes to be used as anticancerigenic agents. US Paten. » CrossRef » PubMed » Google Scholar

20. Ruiz-Azuara L (1992) Process to obtain new mixed copper amino-acidate complexes from phenylatephenanthroline to be used as anticancerigenic agents. US Patent. » CrossRef » PubMed » Google Scholar

21. Ruiz-Ramírez L, Gasque L, Martínez A, Moreno-Esparza R, Solans X (1992) Copper (II) Hydrate mixed chelate complexes, Part III. Structures of (L-alaninato) (aqua) (2, 2´-bypiridine) copper (II) Nitrate Monohydrate and Aqua(2,2´-bipyridine(L-tyrosinato) copper (II) Chloride Trihydrate. Acta Crystallogr C48: 1785-1788. » CrossRef » PubMed » Google Scholar

22. Ruiz-Ramírez L, Gasque L, Martínez A, Moreno-Esparza R, Solans X (1993) Copper (II) Hydrate mixed chelate complexes, Part II. Structures of (L-alaninato) (aqua) (4, 7-diphenyl-1,10-phenanthroline) copper (II) Nitrate Monohydrate and aqua(4,7-dimethyl-1,10-phenanthroline) (glycinato) (nitrato) copper(II) Monohydrate. Acta Crystallogr C49: 890-893. » CrossRef » PubMed » Google Scholar

23. Ruiz-Ramírez L, Gracia-Mora I, Moreno Esparza R, Díaz D, Gasque L, et al. (1991) The antitumor actvity of several transition metal complexes. J Inorg Biochem 43: 615. » CrossRef » PubMed » Google Scholar

24. Reyes L, Fuentes-Noriega I, Ruiz-Ramírez L, Macías L (2003) Development and Validation of a liquid chromatographic method for Casiopeína IIgly® in rat plasma. J Chromatogr B 791: 111-116. » CrossRef » PubMed » Google Scholar

25. Trejo-Solís C, Palencia G, Zúñiga S, Rodríguez-Ropon A, Osorio-Rico L, et al. (2005) CasIIgly induces Apoptosis in Glioma C6 Cells in Vitro and In Vivo Through Caspase-dependent and Caspase-independent Mechanism. Neoplasia 7: 563-574. » CrossRef » PubMed » Google Scholar

26. Wang T, Guo Z (2006) Copper in Medicine: Homeostasis, Chelation Therapy and Antitumor Drug Design. Curr Med Chem 13: 525-537. » CrossRef » PubMed » Google Scholar

27. Yamauchi O, Odoni A (1985) Structure-stability relationship in ternary copper(II) complexes involving aromatic amines and tyrosine or related amino acids. Intramolecular aromatic ring stacking and its regulation through tyrosine phosphorylation. J Am Chem Soc 107: 5938-5945. » CrossRef » PubMed » Google Scholar