Determination and Pharmacokinetics of a New Diorganotin(IV) Complex Dibutyldi(4-chlorobenzohydroxamato)tin(IV) in Rat Plasma by a High Performance Liquid Chromatographic Method

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Dibutyldi(4-chlorobenzohydroxamato)tin(IV) is a new diorganotin(IV) arylhydroxamate complex with 4-chlorobenzohydroxamic acid as ligand which shows high *in vivo* and *in vitro* antitumor activity. A high performance liquid chromatographic (HPLC) method using a Diamonsil ODS column was first validated in the pharmacokinetic studies in rat plasma. The plasma was deproteinized with methanol that contained acetanilide as the internal standard. The mobile phase was a mixture of methanol and 0.5% trifluoroacetic acid (TFA) in water (30:70) (pH 3.0). The detection wavelength was set at 238 nm. A linear curve over the concentration range 0.1 – 25 µg/ml ($r = 0.9992$) was obtained. The method was used to determine the concentration-time profiles for dibutyldi(4-chlorobenzohydroxamato)tin(IV) in the plasma after a single intravenous dose of 2, 5, and 12 mg/kg to rats. The pharmacokinetics parameter calculations and modeling were carried out using the 3p97 pharmacokinetics software. A nonlinear pharmacokinetics was found in rats at doses from 2 to 12 mg/kg. The results showed that the concentration-time curves of dibutyldi(4-chlorobenzohydroxamato)tin(IV) in rat plasma could be fitted to two-compartment model.

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Introduction

Malignancy is the result of a multiple process including accumulation of mutations and other genetic alterations.¹ Searches for non-platin metal-based antitumor drugs have attracted considerable interest. Diorganotin(IV) complexes are potential antitumor agents mainly active against P₃₈₈ lymphocytic leukemia and other tumors.²⁻⁴ These antitumor agents are actually being studied widely.⁵ Among organotin compounds, dibutyltin(IV) derivatives of hydroxamic acid have received more attention due to their structural and biological importance.⁶⁻⁹

Recently, we reported a series of diorganotin arylhydroxamates which exhibit *in vitro* antitumor activities (against a series of human tumor cell lines) which, in some cases, are identical to, or even higher than, that of *cisplatin*.^{10,11} However, their antitumor molecular action mechanisms are still unclear. In order to study the precise action mechanism of the antitumor organotin compounds, one should first elucidate their fates in a body, including their absorption, distribution, metabolism and elimination. Therefore, it is essential to establish a rapid, sensitive and accurate method to determine the organotin compounds in a body. Several methods have been reported for the determination of organotin compounds, including high performance liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry (HPLC-APCI-MS) method,^{12,13} high performance liquid chromatography-inductively coupled plasma-mass spectrometry (HPLC-ICP-MS) method,¹⁴⁻¹⁷ gas chromatographic (GC) method,^{18,19} gas

chromatography-molecularly imprinted polymer-atomic emission detection (GC-MIP-AED)²⁰ method, gas chromatography-inductively coupled plasma-mass spectrometry (GC-ICP-MS) method²¹ and gas chromatography-mass spectrometry (GC-MS)²² methods. However, the disadvantages of these methods are obvious.^{12,18} For examples, GC methods require complicated pretreatments for the samples and seem to be unsuitable for quantitative determination in rat plasma because only a small amount of blood is normally used in pharmacokinetic studies. The HPLC-MS and GC-MS methods are not common due to the high analysis cost and the expensive apparatus required. For medical care, practical, robust, simple and efficient analytical methods are needed. In this study, a classic liquid extraction with methanol, a directly UV detecting HPLC method, successfully achieved the goals of sensitivity, specificity and accuracy. This HPLC with UV detection method is more useful for medical experiments. So far, to the best of our knowledge, no method has been reported for the determination of diorganotin compounds by the HPLC method with UV detection in the pharmacokinetic studies in rat plasma.

Dibutyldi(4-chlorobenzohydroxamato)tin(IV) (Fig. 1) is a potential candidate for the clinical application due to its high *in vivo* and *in vitro* activity against gastric and liver carcinomas.¹¹ But up to now, it still does not get into clinical trail due to its

Fig. 1 Chemical structure of dibutyldi(4-chlorobenzohydroxamato)tin(IV).

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relative high toxicity and the remaining necessary tests. The pharmacokinetic studies of the complex are essential in order to elucidate the toxic mechanism. However, nothing is known about the pharmacokinetic behavior of antitumor diorganotin(IV) compounds in a body. Therefore, in this research, we chose dibutyl-di(4-chlorobenzohydroxamato)tin(IV) as a typical antitumor diorganotin arylhydroxamate to develop a simple, sensitive and specific HPLC assay for its quantitative determination in rat plasma and to investigate its preliminary pharmacokinetic properties.

Experimental

Materials and reagents

Dibutyl-di(4-chlorobenzohydroxamato)tin(IV) used in analysis was synthesized by the same method as that described earlier.¹⁰ Its purity was 99.7%. Acetanilide (internal standard, I.S.) was purchased from Sigma Laboratories (St. Louis, MO). Methanol and trifluoroacetic acid (TFA) were obtained from Tianjin Siyou Co. Ltd. (Tianjin, China) and from the Shanghai Biotechnology Institute (Shanghai, China), respectively. All solvents used were of HPLC grade.

Instrumentation and chromatographic conditions

The HPLC analysis was carried out using a Waters 2695 HPLC system (Waters Associates, Milford, MA) which consisted of a photodiode array detector, an autosampler and a degasser. The apparatus was interfaced to a DELL PC compatible computer using Empower Pro software. The autosampler was cooled to 10°C. The column was maintained at room temperature. Chromatographic separation of dibutyl-di(4-chlorobenzohydroxamato)tin(IV) and the I.S. was achieved on a Diamonsil C₁₈ column (200 mm × 4.6 mm, 5 µm) from Dikma Technologies (Beijing, China) protected by a Diamonsil C₁₈ guard column.

The mobile phase for HPLC analysis consisted of 0.5% TFA in water (solvent A)/methanol (solvent B) with a flow rate of 1.0 ml/min. A sample volume of 50 µl was injected. The HPLC isocratic mode was 30% solvent B at 30 min (v/v). Prior to use, the mobile phase was filtered through a 0.45-µm hydrophilic membrane filter. Detection was performed at a wavelength of 238 nm at room temperature. Under the chromatographic condition mentioned above, dibutyl-di(4-chlorobenzohydroxamato)tin(IV) and acetanilide could be separated completely in the chromatograms ($R > 1.5$), and there were no endogenous interferences with the chromatographic peak of acetanilide. Besides, the retention time of acetanilide ($t_R = 13$ min) was very suitable as I.S. compared to that of dibutyl-di(4-chlorobenzohydroxamato)tin(IV) ($t_R = 15$ min); so, in this study, acetanilide was used as the I.S.

Preparation of plasma samples

Blood samples collected from rat blood plasma were immediately transferred to 1.5 ml heparinized microcentrifuge tubes from fossa orbitalis of rats, and then processed for plasma by centrifugation. The supernatant plasma (0.2 ml) was then vortex-mixed with methanol (0.4 ml) containing acetanilide (25.0 µg/ml) as internal standard (I.S.) for 30 s and allowed to stand for 10 min. After vortex-mixing, the mixture was centrifuged at 14000 rpm for 5 min at room temperature to separate precipitated proteins. Fifty microliters of the supernatant solution of methanol layer were directly injected into the chromatography. The same sample processing was also applied to the recovery and to the precision study in plasma. All

harvested samples stored at -4°C were brought to room temperature before use and analyzed within one week. No significant differences were found between the samples stored at -4°C and those stored at -20°C.

Bioanalytical method validation

Preparation of stocks, calibration standards and quality control samples. A stock solution of dibutyl-di(4-chlorobenzohydroxamato)tin(IV) was prepared in methanol at the concentration of 600 µg/ml and was further diluted in methanol to make working standards. Each solution of I.S. was prepared and diluted to 25 µg/ml with methanol. The linearity of the HPLC method for the determination of dibutyl-di(4-chlorobenzohydroxamato)tin(IV) was evaluated by a calibration curve in the range of 0.1 - 25 µg/ml. Calibration standard samples were prepared by adding different concentrations of the standards of dibutyl-di(4-chlorobenzohydroxamato)tin(IV) and 200 µl of the I.S. working solution to the blank plasma. The final concentrations of dibutyl-di(4-chlorobenzohydroxamato)tin(IV) were 0.1, 0.5, 1.0, 2.5, 5.0, 10.0 and 25.0 µg/ml. The least squares linear regression analysis was used to determine the slope, intercept and correlation coefficient. The calibration curve requires a correlation coefficient (r^2) of 0.99 or better. Three concentrations (0.8, 4 and 20 µg/ml) were selected as quality control (QC) samples for assay validation. These samples were prepared in a manner similar to that used for preparation of the calibrator samples.

Recovery. The recoveries of the dibutyl-di(4-chlorobenzohydroxamato)tin(IV) samples were calculated by comparing the peak areas between the dibutyl-di(4-chlorobenzohydroxamato)tin(IV) plasma samples deproteinized with methanol and the samples in which dibutyl-di(4-chlorobenzohydroxamato)tin(IV) was spiked directly in methanol at the same concentrations. The recoveries at three QC levels (the standard concentrations were 0.4, 8 and 20 µg/ml, respectively) of the compound in plasma were examined at least five times.

Accuracy and precision. To evaluate the accuracy and precision, we used at least five QC samples of three different concentrations of dibutyl-di(4-chlorobenzohydroxamato)tin(IV). The samples were processed and injected on a single day (intra-day) and at different days (inter-day) in rat plasma. The variability of dibutyl-di(4-chlorobenzohydroxamato)tin(IV) determination was expressed as the relative standard deviation (RSD) which should be ≤15% at all concentrations of dibutyl-di(4-chlorobenzohydroxamato)tin(IV).

Quantification and limit of detection. To determine the limit and quantification of detection, we prepared dilutions of 1, 2.5, 5, 10, 15, 20 and 30 ng/ml dibutyl-di(4-chlorobenzohydroxamato)tin(IV) in plasma using a solution of 1.0 µg/ml dibutyl-di(4-chlorobenzohydroxamato)tin(IV) in plasma. The results were evaluated by analyzing each plasma sample spiked with the analyte at a final concentration at which the signal-to-noise ratio (S/N) was 10 and 3.

Selectivity. The selectivity of the method was determined by examining the separation of dibutyl-di(4-chlorobenzohydroxamato)tin(IV) from the endogenous plasma constituents in the blank plasma. The effect of I.S. was also investigated. Solutions of dibutyl-di(4-chlorobenzohydroxamato)tin(IV) in methanol and in plasma were injected under the same conditions, and the retention times of dibutyl-di(4-chlorobenzohydroxamato)tin(IV) and of I.S. were determined.

Stability studies. To investigate the stability of dibutyl-di(4-chlorobenzohydroxamato)tin(IV), we determined the freeze-thaw stability of the analyte over three freeze-thaw cycles. In each freeze-thaw cycle, the samples were frozen and stored at -80°C

for 10 days, then thawed at room temperature. The stability of the fresh plasma samples was tested after keeping the samples at -4 , -20°C and room temperature for 72 h. The stability of deproteinized samples at 10°C in the autosampler was evaluated up to 24 h.

Pharmacokinetic study. The plasma pharmacokinetics of dibutyl-di(4-chlorobenzohydroxamato)tin(IV) was studied in six wistar rats with body weight 180–220 g (three males and three females, respectively). Wistar rats were from the Laboratory Animal Center of Shanxi Medical University. They were fasted for 12 h prior to and during the experiments, while they were allowed free access to water. Dibutyl-di(4-chlorobenzohydroxamato)tin(IV) aqueous solution was then intravenously administered to rats at different doses of dibutyl-di(4-chlorobenzohydroxamato)tin(IV) (2, 5, and 12 mg/kg) by direct injection into a lateral tail vein, with a duration of infusion less than 1 min. Heparinized blood samples (0.5 ml) were collected at 0, 1, 3, 5, 10, 20, 45, 60, 120 and 240 min after injection. Six rats were used for each time point. After each sampling, the removed volume of blood was supplemented with an equal volume of sodium chloride. The blood samples were immediately centrifuged and the resulting plasma was prepared according to the procedure given for the calibrators. Pharmacokinetic analysis of dibutyl-di(4-chlorobenzohydroxamato)tin(IV) concentrations in plasma was performed using two-compartment model methods via the 3p97 software package (Chinese Pharmacology Society).

Results

Method development

A deproteinized method to detect dibutyl-di(4-chlorobenzohydroxamato)tin(IV) in plasma has been first developed in this paper. Diverse proportional solvents (methanol and acidified water) were selected in the deproteinizing process. Although a 500- μl sample spiked with 100 μl methanol could produce the minimal dilution, optimum peak shape and an increase of detector sensitivity accordingly, the recovery was unsuitable. However, the plasma deproteinized with double volume of methanol produced a similar optimum peak shape along with the satisfactory recovery. The chromatograms of a blank plasma sample, a dibutyl-di(4-chlorobenzohydroxamato)tin(IV) quality control sample (3 $\mu\text{g}/\text{ml}$) and a rat plasma sample after dosing with dibutyl-di(4-chlorobenzohydroxamato)tin(IV) (5 mg/kg) are shown in Fig. 2. There were no endogenous interfering peaks with dibutyl-di(4-chlorobenzohydroxamato)tin(IV) and the I.S. in the rat plasma. The retention times of dibutyl-di(4-chlorobenzohydroxamato)tin(IV) and the I.S. were 14.8 and 12.9 min, respectively.

Linearity, quantification and limit of detection

The stock solution of dibutyl-di(4-chlorobenzohydroxamato)tin(IV) was diluted to give a series of standard solutions (0.1, 0.5, 1, 2.5, 5, 10 and 25 $\mu\text{g}/\text{ml}$) for the calibration curves of dibutyl-di(4-chlorobenzohydroxamato)tin(IV) in rat plasma. The calibration curve of dibutyl-di(4-chlorobenzohydroxamato)tin(IV) was linear in the range of 0.1–25 $\mu\text{g}/\text{ml}$. The regression equation was $y = 19.397x + 0.0396$ ($r = 0.9992$, $n = 7$), where y represented the peak-area ratio of dibutyl-di(4-chlorobenzohydroxamato)tin(IV) to the I.S. in rat plasma and x was the concentration of dibutyl-di(4-chlorobenzohydroxamato)tin(IV). The quantification and the limit of detection were 50 and 10 ng/ml, respectively.

Fig. 2 Represent chromatograms of dibutyl-di(4-chlorobenzohydroxamato)tin(IV) and acetanilide (I.S.) in plasma sample. (a) Blank plasma, (b) plasma spiked with dibutyl-di(4-chlorobenzohydroxamato)tin(IV) at a concentration of 3 $\mu\text{g}/\text{ml}$ and acetanilide (I.S., 8 $\mu\text{g}/\text{ml}$), (c) a rat plasma sample obtained at 3 min after intravenous administration of dibutyl-di(4-chlorobenzohydroxamato)tin(IV) at a dose 5 mg/kg. 1, I.S. (acetanilide); 2, dibutyl-di(4-chlorobenzohydroxamato)tin(IV).

Recoveries

The recovery of the assay was assessed by comparing the peak-area ratios (analyte/I.S.) obtained from spiked plasma samples of three dibutyl-di(4-chlorobenzohydroxamato)tin(IV) standard concentrations (0.4, 8, 20 $\mu\text{g}/\text{ml}$) with the peak-area ratios (analyte/I.S.) for the samples containing the equivalent dibutyl-di(4-chlorobenzohydroxamato)tin(IV) and I.S. which were directly dissolved in methanol. The recoveries of the compound from rat plasma are shown in Table 1, the mean extraction recovery and the relative standard deviation (RSD) of dibutyl-di(4-chlorobenzohydroxamato)tin(IV) at three different concentrations from rat plasma were 97.6 ($n = 15$) and 4.5%, respectively.

Precision and accuracy

The precision and accuracy of this method were evaluated by assaying each low, middle and high concentration QC sample. The reproducibility of the method was assessed by examining both intra- and inter-day variance. The data showed that the relative standard deviation (RSD) values of the intra-day and the inter-day for rat plasma samples ranged from 1.8–5.7% and 0.4–4.3%, respectively. The relative standard deviations of the

Table 1 Recovery of the assay for determining the concentrations of dibutyl(4-chlorobenzohydroxamato)tin(IV) ($n = 5$)

Concentration/ $\mu\text{g ml}^{-1}$	Recovery, mean $\pm$ SD%	RSD, %
0.8	97.4 $\pm$ 6	6.4
4	98.1 $\pm$ 2	1.6
20	97.2 $\pm$ 5	4.2

Table 2 Stability results of dibutyl(4-chlorobenzohydroxamato)tin(IV) at different conditions ($n = 5$)

Stability	Concentration/ $\mu\text{g ml}^{-1}$	RSD, %
Concentration of fresh preparation	12.00	1.1
Three freeze and thaw cycles	10.99	3.3
Stability for 72 h at -4°C	11.85	2.5
Stability for 72 h at -20°C	12.33	2.4
Stability for 72 h at room temperature	10.79	2.6
Autosampler stability for 24 h at 10°C	11.21	2.2

three QC samples were less than 6%. These validations demonstrated the reliability of the assay.

Selectivity and retention time

Figures 2(a), 2(b) and 2(c) show the chromatograms of blank rat plasma, plasma spiked with the standards and plasma sample from the dibutyl(4-chlorobenzohydroxamato)tin(IV) pharmacokinetic applicability study. The results show that there were no endogenous interfering peaks with the internal standard (I.S., acetanilide) and dibutyl(4-chlorobenzohydroxamato)tin(IV) in the rat plasma. The retention times of dibutyl(4-chlorobenzohydroxamato)tin(IV) and I.S. were approximately 15 and 13 min, respectively.

Stability studies

Stability of dibutyl(4-chlorobenzohydroxamato)tin(IV) during storage and processing was checked using quality control samples. The dibutyl(4-chlorobenzohydroxamato)tin(IV) and I.S. stock solutions were stable for at least 2 months when stored at 4°C . The deviation of the mean test responses was within $\pm 5\%$ of appropriate controls in all stability tests of dibutyl(4-chlorobenzohydroxamato)tin(IV) in rat plasma. After three freeze-thaw cycles, the peak area ratio changes of dibutyl(4-chlorobenzohydroxamato)tin(IV) were less than 4%. The analyte was stable in the matrices at -4 , -20°C and room temperature for 72 h without significant degradation ($<3\%$). The run-time stability study showed that dibutyl(4-chlorobenzohydroxamato)tin(IV) in deproteinized rat plasma was stable at 10°C for up to 24 h compared with 0 h ($<3\%$). These results suggested that the rat plasma samples containing dibutyl(4-chlorobenzohydroxamato)tin(IV) can be handled under normal laboratory conditions without significant loss of the compound. All stability results are summarized in Table 2.

Pharmacokinetic applicability

The developed RP-HPLC analytical method has been successfully used for the pharmacokinetic study after a single intravenous administration of dibutyl(4-chlorobenzohydroxamato)tin(IV) in rats. After tail vein injection, dibutyl(4-chlorobenzohydroxamato)tin(IV) in plasma were determined for a series of time points within 4 h. The mean plasma concentration-time

Fig. 3 Mean plasma concentration-time profiles of dibutyl(4-chlorobenzohydroxamato)tin(IV) in rats after intravenous administration of 2, 5 and 12 mg/kg of dibutyl(4-chlorobenzohydroxamato)tin(IV). Each point represents the mean concentration of six rats.

curves of dibutyl(4-chlorobenzohydroxamato)tin(IV) after intravenous administration at doses of 2, 5 and 12 mg/kg in rats are shown in Fig. 3, and the mean pharmacokinetic parameters (mean $\pm$ SD) are summarized in Table 3. The plasma dibutyl(4-chlorobenzohydroxamato)tin(IV) concentration-time curves were fitted to a two-compartment open model. Table 3 shows that there was significant difference in the elimination half-life $t_{1/2\beta}$ (64.67, 56.80, 220.60 min, respectively) when the injection dose of dibutyl(4-chlorobenzohydroxamato)tin(IV) was increased from 2 to 12 mg/kg. These results suggested that the pharmacokinetics of the complex is a nonlinear process. The nonlinear increasing of $t_{1/2\beta}$ with the increased dosage suggested the saturated elimination of dibutyl(4-chlorobenzohydroxamato)tin(IV) and the nonlinear pharmacokinetics in rats from 2 to 12 mg/kg.

The AUC were calculated to be 78.74, 123.38, 341.01 $\text{mg kg}^{-1} \text{min}^{-1}$ at doses of 2, 5 and 12 mg/kg, respectively. The $t_{1/2\alpha}$ were approximately 2.36, 1.96, 2.11 min after three dosages, indicating that dibutyl(4-chlorobenzohydroxamato)tin(IV) distributed very quickly in rats. The value of V_d after three dosages were 0.835, 0.698 and 0.610 L kg^{-1} , respectively. There were no significant differences in all pharmacokinetic parameters between male and female rats at dose of 2, 5 and 12 mg/kg. The RP-HPLC method satisfied the requirement of this study and demonstrated its general suitability for pharmacokinetics studies of dibutyl(4-chlorobenzohydroxamato)tin(IV) in rats.

Discussion

HPLC analysis for amphoteric, polar substances with low wavelength ultraviolet absorption is always associated with some difficulties, especially when high sensitivity is required. In some cases, special analysis columns, the soy protein extract (SPE) or ultrafiltration were used to decrease or remove the endogenous interfering substances to a maximal extent. HPLC-MS method¹²⁻¹⁷ are also used to increase the sensitivity and reduce the interferences. The highest sensitivity could be achieved by using a LC-MS method, but the HPLC-MS methods are not common due to the high analysis cost and the expensive apparatus required. The results showed that this classic liquid extraction HPLC with UV detecting method was sensitive (the limit of detection was 10 ng/ml) enough for pharmacokinetics studies of dibutyl(4-chlorobenzohydroxamato)tin(IV) in rats and did not require any forms of analyte derivatization or special columns or instruments. The most important factor for achievement of high sensitivity was the clean background on the chromatograms. The chromatographic condition of the acidic mobile phase showed strong retention effect. This effect, combined with carefully adjusted ratio of 0.5% trifluoroacetic

Table 3 Mean pharmacokinetic parameters in rats after intravenous administration of 2, 5 and 12 mg/kg of dibutyl-di-(4-chlorobenzohydroxamato)tin(IV) (mean $\pm$ SD, $n = 6$)

Parameter (unit)	Dosage/mg kg ⁻¹		
	2	5	12
A/min^{-1}	0.62 ± 0.68	0.41 ± 0.14	0.39 ± 0.18
$B/\text{mg kg}^{-1}$	1.25 ± 1.36	1.79 ± 1.09	3.36 ± 4.03
β/min^{-1}	0.03 ± 0.04	0.02 ± 0.01	0.02 ± 0.04
$V_d/L \text{ kg}^{-1}$	0.84 ± 0.37	0.70 ± 0.30	0.61 ± 0.29
$t_{1/2\alpha}/\text{min}$	2.36 ± 1.62	1.96 ± 0.86	2.11 ± 0.78
$t_{1/2\beta}/\text{min}$	64.67 ± 49.40	56.80 ± 33.19	220.60 ± 182.09
K_{21}/min^{-1}	0.42 ± 0.73	0.10 ± 0.06	0.08 ± 0.10
K_{10}/min^{-1}	0.06 ± 0.04	0.10 ± 0.09	0.11 ± 0.13
K_{12}/min^{-1}	0.17 ± 0.09	0.26 ± 0.12	0.20 ± 0.08
$AUC/\text{mg kg}^{-1} \text{ min}^{-1}$	78.74 ± 67.37	123.38 ± 30.62	341.01 ± 108.89
$CL(s)/\mu\text{g ml}^{-1} \text{ mg kg}^{-1}$	0.044 ± 0.023	0.043 ± 0.01	0.044 ± 0.03

acid (TFA) water to methanol, resulted in a clean baseline for the peaks of the studied compound. Thus, a high signal/noise ratio was achieved, which constituted the base for the high sensitivity. The second factor was the high recovery of dibutyl-di-(4-chlorobenzohydroxamato)tin(IV) from plasma during the extraction process. Methanol was used not only for deproteination, but also as extractive solvent for assay. So, the high recovery of dibutyl-di-(4-chlorobenzohydroxamato)tin(IV) (97.4, 98.1 and 97.2% for three determinations, respectively) was obtained. During the process of method development, it was discovered that the dibutyl-di-(4-chlorobenzohydroxamato)tin(IV) spiked with aqueous I.S. showed a symmetric single peak in the chromatograms at a pH around 3.0. With high sensitivity, small sample requirement and simple sample treatment procedures, this method was successfully applied to the analysis of rat plasma samples.

Conclusion

In this paper, a new, economical, easy to perform and sensitive method for determination of dibutyl-di-(4-chlorobenzohydroxamato)tin(IV) in rat plasma is reported. The method was validated for linearity, specificity, accuracy, precision and recovery and good results were obtained. This is also the first case reported that a HPLC method with UV detection was used for the pharmacokinetic study of a diorganotin antitumor compound *via* intravenous administration and could be successfully used for the analysis of large numbers of rat plasma samples. The results of preliminary pharmacokinetic studies indicated that dibutyl-di-(4-chlorobenzohydroxamato)tin(IV) showed non-linear pharmacokinetics in the studied dose ranges in rats. Moreover, the present studies showed that dibutyl-di-(4-chlorobenzohydroxamato)tin(IV) distributed and was eliminated very quickly, indicating that dibutyl-di-(4-chlorobenzohydroxamato)tin(IV) might accumulate in certain organ or had been metabolized in the plasma. The mechanism is still ambiguous and further

researches are required.

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