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Determination of 2-*n*-propylquinoline in mouse plasma and liver by high-performance liquid chromatography

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Abstract

A high-performance liquid chromatographic method was developed for the specific determination of 2-*n*-propylquinoline, a new anti-leishmaniasis drug, in plasma and liver homogenates of mice. 2-*n*-Propylquinoline was extracted with methyl-*tert*.-butyl ether with quinoline as internal standard. Separation was carried out using a Nucleosil C_{18} column. The mobile phase consisted of methanol–0.005 *M* ammonium acetate buffer (60:40) at pH 5.5 and 8 for plasma and liver homogenates, respectively. Detection was monitored at 233 nm. The method was validated and shown to be accurate and precise for plasma and liver homogenates. Extraction yield was 96% in plasma and 81% in liver homogenates. This method was used to determine the pharmacokinetic profile of 2-*n*-propylquinoline following oral administration to mice.

Keywords: 2-*n*-Propylquinoline

1. Introduction

Visceral leishmaniasis (VL) is a widespread disease caused by *Leishmania donovani*, *L. infantum* and *L. chagasi*. 2-*n*-Propylquinoline is an alkaloid extracted from *Galipea longiflora* of the *Rutaceae* family [1]. This plant was used for the treatment of cutaneous leishmaniasis topically by Bolivians [2,3]. Among the 20 or so 2-substituted quinoline components extracted and identified from this plant, 2-*n*-propylquinoline (Fig. 1) has shown the best in vivo activity against *L. donovani*. Oral administration of 0.54 mmol/kg of 2-*n*-propylquinoline once daily for 10 days to *L. donovani*-infected mice was able to suppress parasite burdens in the liver by 99.9% [4]. It could be an alternative to actual therapies (i.e.,

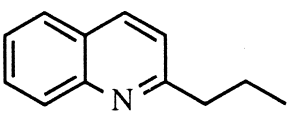

Fig. 1. Chemical structure of 2-*n*-propylquinoline.

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aromatic diamidines, amphotericin B and pentavalent antimony).

The aim of this study was to develop an extraction procedure and high-performance liquid chromatography (HPLC) method for the simple determination of 2-*n*-propylquinoline in mouse plasma and liver homogenates to gain information on its tissue distribution.

2. Experimental

2.1. Chemicals

2-*n*-Propylquinoline was synthesized according to the method of Munos et al. [5]. Quinoline and methyl-*tert*.-butyl ether (MTBE) were purchased from Sigma–Aldrich (St. Quentin Fallavier, France). Sodium hydroxide and hydrochloric acid were purchased from Labosi (Oulchy-le-château, France). Ammonium acetate was from Prolabo (Paris, France) and HPLC grade solvents (methanol, ethanol) were from Carlo Erba (Val de Reuil, France).

2.2. Materials and chromatographic conditions

The LC system consisted of a Shimadzu LC-6A pump (Touzart et Matignon, Les Ulis, France), a Wisp 717+ autosampler (Waters-Millipore, Saint Quentin en Yvelines, France), a spherisorb ODS2 5 μm, 150×4.6 mm I.D. column (Shandon, Eragny, France), a SPD-6A UV detector and a C-R6A integrator (both from Touzart et Matignon).

The mobile phase was a mixture of methanol–0.005 *M* ammonium acetate (60:40, v/v). The flow-rate was 1 ml/min. The pH of the ammonium acetate was adjusted to 5.5 for plasma analyses and to 8 for liver samples. On average 20 μl were injected into the HPLC system. The detector wavelength was set at 233 nm.

2.3. Preparation of stock and working solutions

Stock solutions (100 mg/l) of 2-*n*-propylquinoline and quinoline were prepared in ethanol and proved to be stable for at least six months when stored at −20°C.

2.4. Animals

Female CDI mice Charles River (France) were force-fed with a single dose of 2-*n*-propylquinoline at 0.54 mmol/kg. A volume of 0.1 ml was administered per 20 g of total body mass. Three animals were sacrificed per time point after 0.5, 1, 1.5, 2, 4 and 6 h. Blood was collected into heparin-coated tubes and the plasma was obtained by centrifugation. The liver was removed from each mouse and crushed in liquid nitrogen (Spex 6700, Bioblock Scientific, Ilkirch, France). All samples were frozen at −20°C until analysis.

2.5. Extraction procedures

2.5.1. Plasma

Fifty μl of quinoline (internal standard 1 mg/l in ethanol) and 200 μl of 6 *M* sodium hydroxide were added to 200 μl of plasma in polypropylene tubes. After vortexing for 30 s, 2 ml of MTBE were added before rotative agitation for 10 min. After centrifugation for 5 min at 1600 *g*, the organic layer was separated, acidified with 50 μl of 2 *M* hydrochloric acid and dried under a stream of nitrogen at room temperature. The residue was dissolved in 100 μl of 0.1 *M* methanol–ammonium acetate, pH 5.5 (60:40, v/v).

2.5.2. Liver tissue

Fifty μl of quinoline (1 mg/l in ethanol) and 400 μl of 3 *M* sodium hydroxide were added to 100 mg of crushed liver in polypropylene tubes. After vortexing for 30 s and exposure to ultrasonication for 10 min, 2 ml of MTBE were added before rotative agitation for 15 min. After centrifugation for 5 min at 1600 *g*, the organic layer was separated, acidified with 50 μl of 1 *M* hydrochloric acid and dried under a stream of nitrogen at room temperature. The residue was dissolved in 200 μl of 0.1 *M* methanol–ammonium acetate, pH 5.5 (60:40, v/v).

2.6. Recovery

The recovery was determined in plasma and liver tissue. One calibration curve was run for each type of sample. Peak areas of the calibration curve samples were compared to those obtained from direct

injection of ethanol solutions corresponding to the same concentrations. For each sample, peak area ratios for the extracted versus the non-extracted 2-*n*-propylquinoline were calculated and mean values were used to calculate the average recovery.

2.7. Calibration

Seven standards within the plasma concentration range (0.05 to 1 mg/l) or five standards within the liver tissue concentration range (0.2 to 8 μg/g) were prepared by adding the appropriate amounts of a 1 mg/l or 10 mg/l 2-*n*-propylquinoline ethanol solution. These standards were extracted as described above and the standard curves plotted as peak area ratios of 2-*n*-propylquinoline to the internal standard.

2.8. Precision and accuracy

The within-day precision was assessed by extracting and analyzing fives times in one day plasma and liver samples spiked with 2-*n*-propylquinoline at two different concentrations. To determine the between-day precision and accuracy, a calibration curve for plasma and liver samples was run on five different days.

To obtain the within-day and between-day coefficients of variation (C.V.s), mean and standard deviations were calculated for each series of analyses.

Accuracy was assessed by expressing the mean assayed concentrations as a percentage of the nominal concentrations.

3. Results and discussion

The absence of any previous analytical method for the determination of 2-*n*-propylquinoline, a original drug, led us to look for methods applied to quinoline derivatives such as quinine, quinidine and chloroquine. Most of them [6–9] used reversed-phase HPLC. A C_{18} column with an ammonium acetate–methanol mobile phase was also used. Drug ionisation is a critical parameter which reduces the efficiency of basic drug separation. Also, the influence of the pH of the aqueous phase on retention times and separation efficiency was studied. pH values of 3 and 5, 6, 7 were obtained using acetic acid and aceto–acetate buffer solutions, respectively. A pH of 5 gave a capacity factor (k') of 12 with an asymmetric factor of 8.3%. The pK_a of 2-*n*-propylquinoline appears to lie between 3 and 5 (k'=8.8, 12, 12 and 12.5 for pH=3, 5, 6 and 7, respectively). Under these conditions, the pH of the mobile phase (set at 5.5) was higher than the pK_a of the molecule (pK_a< 5). Due to the presence of an impurity in the liver tissue, it was necessary to change the pH of the ammonium acetate buffer from 5.5 to 8. Methanol percentage of the mobile phase was also determinant on the chromatographic separation of 2-*n*-propylquinoline and internal standard: an increase in methanol from 50% to 75% was associated with a 2-*n*-propylquinoline decrease in k' from 96.5 to 10.3.

Finally, a mobile phase composed of methanol–0.005 *M* ammonium acetate, pH 5.5 (60:40, v/v) allowed a good separation without interferences from plasma (Fig. 2).

Due to the presence of a quinoline nucleus in the 2-*n*-propylquinoline, it fluorescence spectrum was studied in order to improve sensitivity and specificity. Nevertheless, UV detection was chosen because of the inability to obtain a fluorescence spectrum under the selected chromatographic conditions. The influence of pH on the absorbance of 2-*n*-propylquinoline was evaluated at three pH values (3, 7 and 10). No bathochromic or hypsochromic effects were detected, therefore the wavelength was set at 233 nm.

3.1. Recovery

Due to the volatility of the 2-*n*-propylquinoline free base, the organic phase was acidified with hydrochloric acid to minimise evaporation during the extraction procedure. To dissolve the residue after evaporation, the ionic strength of the mobile phase used was increased [methanol–0.1 *M* ammonium acetate (60:40, v/v)]. Under these conditions the recovery yields of 2-*n*-propylquinoline from plasma and liver tissue were 96% (S.D. ±6%) and 81% (S.D. ±15%), respectively.

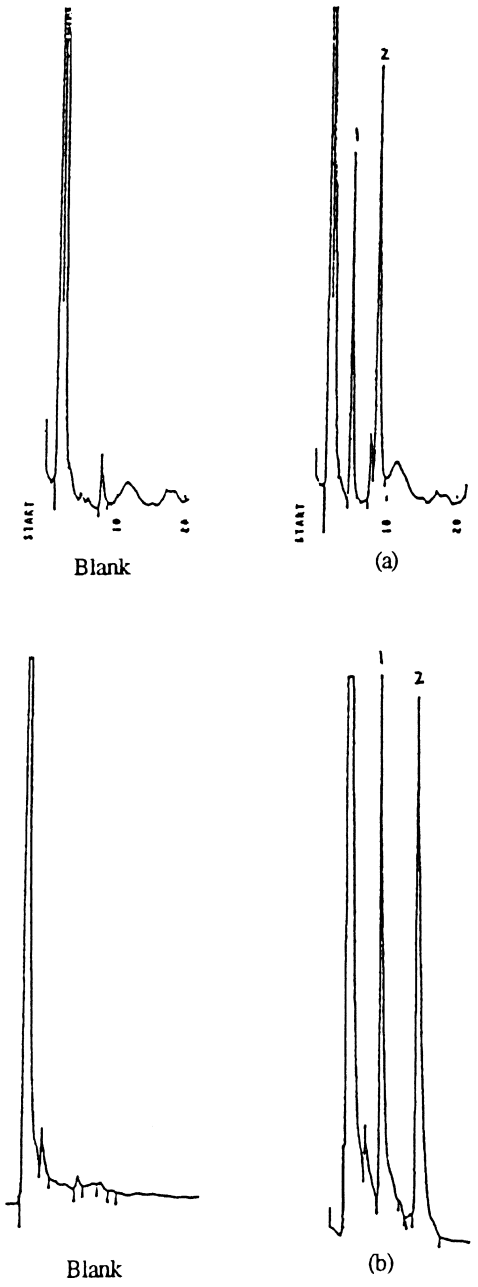

Fig. 2. Chromatograms of plasma and liver homogenate samples spiked at respectively, 0.5 mg/l (a) and 8 μg/g (b) of 2-*n*-propylquinoline, respectively. The retention time for 2-*n*-propylquinoline (2) is 8.7 min in plasma and 8.8 min in liver homogenates. The retention time for internal standard (1) is 4.9 min in both matrices.

3.2. Linearity, accuracy and precision

The calibration curves for 2-*n*-propylquinoline were linear in the range of 0.05–1 mg/l in plasma and 0.2–8 μg/g in liver homogenate. Typical calibration curve equations were $y=2.1001x+0.0034$ mg/l ($r=0.9967$) in plasma and $y=0.9150x-0.0110$ μg/g ($r=0.9840$) in liver homogenates.

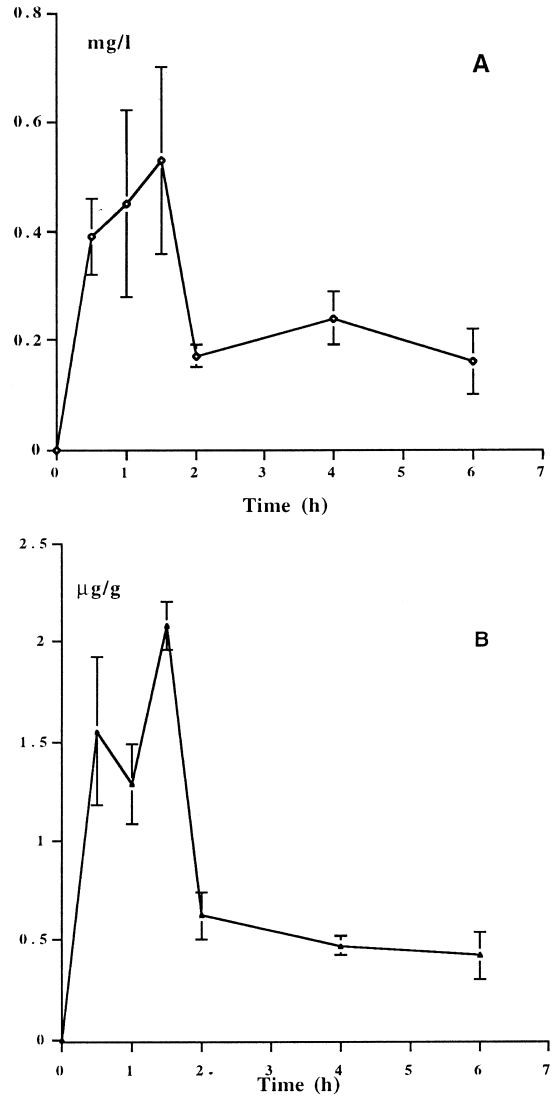

Fig. 3. 2-*n*-Propylquinoline levels and S.E.M. at time points in plasma (A) and liver homogenate (B).

Table 1
Within-day precision and accuracy data for 2-*n*-propylquinoline in plasma and liver homogenates

Sample matrix	Concentration added	Mean concentration found (n=5)	S.D.	C.V. (%)	Accuracy (%)
Plasma (mg/l)	0.1	0.095	0.01	15.1	−5.5
	0.8	0.88	0.07	8.5	+1.0
Liver homogenate (μg/g)	1	1.05	0.09	8.8	+5.0
	6	6.44[a]	1.0[a]	14.2[a]	+7.3[a]

[a] n=4.

Table 2
Between-day precision and accuracy data for 2-*n*-propylquinoline in plasma and liver homogenate

Sample matrix	Concentration added	Mean concentration found (n=5)	S.D.	C.V. (%)	Accuracy (%)
Plasma (mg/l)	0.05	0.047	0.008	18.3	−6.0
	0.1	0.101	0.008	7.8	+1.0
	0.2	0.199	0.011	6.0	−0.5
	0.5	0.514	0.034	6.6	+2.8
	0.8	0.793	0.053	6.7	−0.9
	1	1.001	0.044	4.4	+0.1
Liver homogenate (μg/g)	0.2	0.258	0.066	18.3	+29
	0.5	0.537	0.082	15.2	+7.4
	2	2.042	0.333	16.3	+2.1
	4	3.671	0.516	14.0	−8.2
	8	8.228	0.938	11.4	+2.9

3.2.1. Comparison test of intercept with 0

Plasma: mean intercept/S.D. intercept $=0.214<t_{34,0.05}=2.034$; liver: mean intercept/S.D. intercept$=0.780<t_{25,0.05}=2.060$.

In both matrices the intercept was not significantly different from 0 ($p<0.05$).

3.2.2. Test of existence of a significant slope

An ANOVA test was used to compare the variations due to the regression and the experimental errors. The $F_{\text{theoretical}}$ and $F_{\text{calculated}}$ were equal to 13.29 and 5937 for plasma and 14.03 and 735 for liver tissue, respectively. In both matrices, F was significative for a linear model ($p<0.001$). So calibration curves plotted as the peak height ratio of 2-*n*-propylquinoline to the internal standard, have been considered linear over the range studied.

The sensitivity of the assay was determined to be 0.05 mg/l in plasma and 0.2 μg/g in liver homogenate. This was calculated by dividing three times the intercept standard deviation by the mean slope of the calibration curves. The performance of the assay was such that it could be applied to pharmacokinetic studies in mice. The result of the precision and accuracy experiments are summarized in Tables 1 and 2. The method is accurate and precise in both matrices with C.V.s below 19%.

3.3. Pharmacokinetic results

Pharmacokinetic results (Fig. 3) show an hepatic distribution which coincides with parasite localization, a $T_{\max}$ about 90 min. In liver, the elimination half-life estimated 4 h post-dose was about 100 min.

In plasma, a second peak around 4 h post-dose was appeared and might characterize a enterohepatic cycle. But, it was not supported by the liver data and must be confirmed in further studies.

4. Conclusions

This HPLC method is the first to allow the simple, sensitive and reliable determination of 2-*n*-propylquinoline in plasma and liver homogenate. It has been used to measure 2-*n*-propylquinoline levels in kidney, spleen and lung in mice, with similar slopes and recoveries as those obtained in liver homogenates (unpublished results). The same method could be suitable for clinical pharmacokinetic studies.

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