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HPLC assay in plasma and preliminary pharmacokinetic study of a quinazolinone derivative with spasmolytic effect

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An analytical method for analysis of 3-(*o*-bromo)-phenyl-2-(2′,3′-dihydroxypropylthio)-4(3*H*)-quinazolinone from rat plasma using HPLC with reversed phase C_{18} and liquid-liquid extraction was developed. This method was used for a pharmacokinetic study in rat.

1. Introduction

3-(*o*-Bromo)-phenyl-2-(2′,3′-dihydroxypropylthio)-4(3*H*)-quinazolinone (BCH) is a new compound described by Jira et al. [1] dealing with its synthesis and its chromatographic behavior on reversed phase C_{18} for purposes of lipophilicity determination. Compounds from this group are optically active substances. Separation of enantiomers using HPLC with chiral stationary phases is described elsewhere [2]. The best separation was achieved using stationary cellulose phases. Effects of mobile phase composition, temperature and substituent position on the separation are discussed.

Spasmolytic properties were found during preliminary pharmaco-dynamic studies. BCH was the most interesting compound. It was necessary to develop a simple and rapid analytical method for the quantitative determination in rat plasma. HPLC was chosen because of its good separation power.

Because the tested substance is new, literature searching was focussed to the structurally similar drug methaqualone. HPLC at a reversed phase was used for screening of methaqualone and other drugs in serum and urine [3, 4], for their simultaneous quantitation [5]. Herre et al. [6] dealt with a shift of retention characteristics in metabolites with regard to the parent drug in a HPLC system with reversed phase C_8 and use of this phenomenon for identification in toxicology. Methaqualone is often used as internal standard – for quantitation of fenitrothione and its metabolites from biological fluids [7], of cocaine and benzoylecgonine from plasma and urine 8 and of kebuzone and its metabolites [9]. Especially solid-phase extraction procedures used for the preparation of biological material samples before analysis [3, 7–9]. Taylor et al. [10] described a modern and not get wide spread method for analysis of methaqualone with preparation and analysis of samples – on-line supercritical fluid extraction-supercritical fluid chromatography (SFE-SFC). Analysis of many drugs including methaqualone using capillary zone electrophoresis (CZE) and micellar electrokinetic capillary chromatography (MEKC) was investigated by Thormann et al. [11].

2. Investigations, results and discussion

The aim of our work was to develop a liquid-liquid extraction procedure to isolate BCH from a biological matrix (plasma). The samples were adjusted as follows: To 25 µl of plasma in a glass-stoppered centrifuge test tube 5 µl of the internal standard solution were added. The plasma sample was extracted with diethylether without pH adjustment, but the recovery was very low (about 45%). An attempt was made to extract BCH with dichloromethane and adjust the pH-value with an addition of borate buffer (pH 9.0). Dichloromethane produces better extraction and borate buffer higher recovery values (Table 1).

Under chromatography conditions described in the Experimental part, BCH and methaqualone (I.S.) were completely separated with retention times of 10.8 and 20.4 min, respectively. No interfering peaks with retention times of analyzed compounds were seen in blank samples of plasma. Fig. 1 shows the representative chromatograms of blank (A), control (B) and dosed (C) rat plasma samples, respectively.

Quantitative determination of BCH was performed. The calibration curve of BCH displayed good linearity over the range examined in plasma. Its regression equation and correlation coefficient are:

$$y = 0.123x + 0.025\,, \qquad r = 0.998\,.$$

Both within-day and day-to-day precision and accuracy of standard curve were examined. Within-day precision was calculated from the analysis of six samples in rat plasma of four concentrations of BCH. Day-to-day reproducibility was investigated during a four-week period. Measured concentrations and coefficients of variation (C.V.) are presented in Table 2; all C.V. values were less than 5%.

Table 1: Recovery of BCH in rat plasma

Added BCH concentration (µg/ml)	Recovery (n = 5) mean ± S.D. (%)
2.0	67.2 ± 4.1
10.0	70.5 ± 2.2
15.0	71.0 ± 2.1

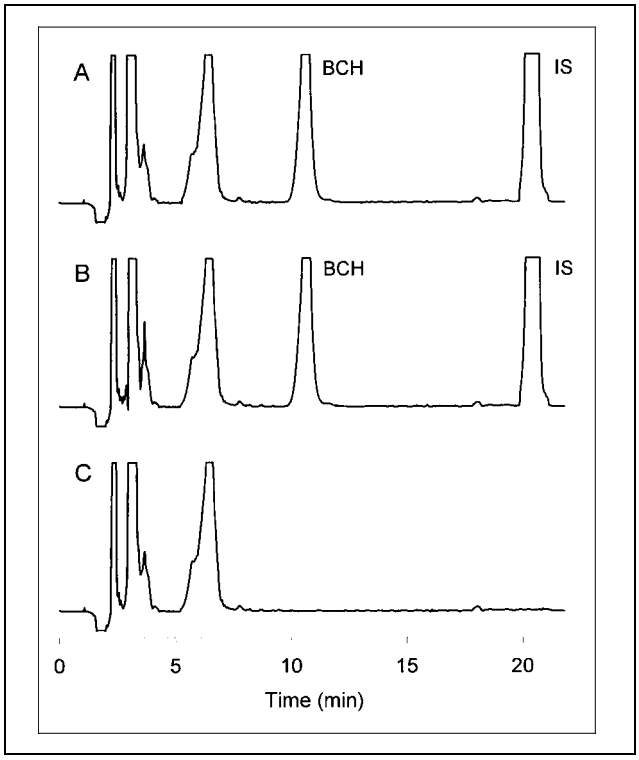

Fig. 1: Chromatograms for BCH (BCH) and internal standard (IS) in rat plasma. A – blank sample, B – control sample spiked with standard solution of BCH (5 μg/ml) and IS, C – 15-min sample from a rat given a single dose of 10 mg/kg BCH

The detection limit for BCH was 0.003 μg/ml in plasma. The limit of quantification was 0.01 μg/ml in plasma.

The plasma concentration-time curve of BCH is depicted in Fig. 2.

Calculated pharmacokinetic parameters of BCH are presented in Table 3.

Elimination of BCH from rat plasma was relatively rapid and efficient. The question of elimination pathways of the agent was not analyzed in this paper.

Table 2: Assay precision and accuracy of the determination of BCH in rat plasma

Conc. added (μg/ml)	Within-day (n = 6)		Day-to-day (n = 12)	
	Concentration found (mean ± S.D.) (μg/ml)	C.V. (%)	Concentration found (mean ± S.D.) (μg/ml)	C.V. (%)
2.00	2.1 ± 0.1	3.3	2.2 ± 0.1	4.2
5.00	4.9 ± 0.1	2.4	5.1 ± 0.2	3.0
10.0	9.8 ± 0.1	1.2	10.3 ± 0.2	2.3
15.0	15.2 ± 0.3	1.8	15.4 ± 0.3	2.2

Table 3: Pharmacokinetic parameters of BCH in rats

Parameter	Value
Plasma clearance (ml/min/kg)	42.1
Distribution volume* (ml/kg)	789
Half-life of elimination (min)	13.0

* volume of total body water

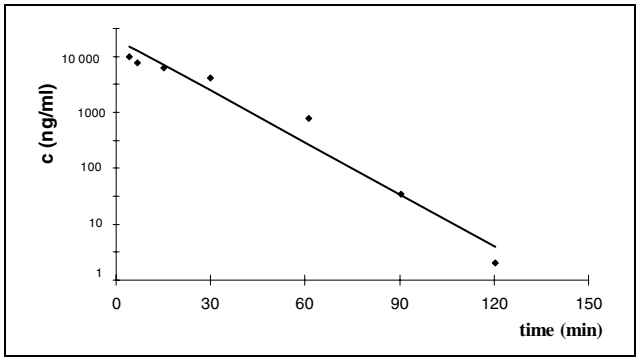

Fig. 2: Plot of the plasma concentration-time curve of BCH in the rat. Experimental points and the calculated course

3. Experimental

3.1. Reagents and chemicals

3-(*o*-Bromo)-phenyl-2-(2′,3′-dihydroxypropylthio)-4(3*H*)-quinazolinone (BCH) was synthesized by the Institute of Pharmacy, Ernst-Moritz-Arndt-University Greifswald (Germany), internal standard (methaqualone) by Léč iva (Prague, Czech Republic). Methanol, dichloromethane, hydrochloric acid and sodium tetraborate were purchased from Lachema (Brno, Czech Republic). All reagents were analytical-reagent grade, methanol was HPLC grade, dichloromethane was distilled. Water was double-distilled in glass. The borate buffer (pH 9.0) used for sample preparation was prepared from sodium tetraborate 0.05 mol/l, adjusted to pH 9.0 with hydrochloric acid 0.1 mol/l.

3.2. Chromatographic system

The HPLC system consisted of a HPP 5001 pump (Laboratorní přístroje, Prague, Czech Republic), HP 1050 UV detector and HP 3394 integrator (Hewlett Packard, U.S.A.). Analyzed samples were introduced onto the column using a C14W injector (Vici, Switzerland) with a 5 μl loop. The analytical steel column contained Separon SGX C_{18} (250 mm × 4 mm I.D., 7 μm, Tessek, Prague, Czech Republic). The mobile phase was a mixture of methanol and water (58 : 42 v/v). It was filtered and helium degassed prior to use. The flow rate was set at 0.7 ml/min. The UV absorbance of the column effluent was monitored at 224 nm.

3.3. Standard solution

Working standards of BCH were prepared by dilutions of 10 mg/ml (in methanol) of the stock standard to aliquot concentrations. The stock solution of the internal standard (0.01 mg/ml) was prepared in methanol.

3.4. Biological sample

A male Wistar rat was placed in plastic restrainers with tails protruding. BCH in a dose of 10 mg/kg was administered into the tail vein in a volume of 0.2 ml. A butterfly cannula was inserted into the lateral opposite vein and at 7 different blood collection times (up to 120 min after administration) the blood samples were withdrawn. During the experiment the rat's tail was warmed at the temperature module at approx. 40 °C.

The plasma concentration-time course of BCH was approximately monoexponential and thus a one-compartment open pharmacokinetic model was employed for the calculation of pharmacokinetic parameters. The decrease of plasma concentrations with time was described by the eq. (1):

$$C = C_1 \exp(-\lambda t) \quad (1)$$

where C is plasma concentration, C_1 is an intercept with ordinate, λ is the disposition rate constant, and t is time.

Pharmacokinetics of BCH was characterized by: distribution volume V, the total plasma clearance value Cl, and half-life of elimination T.

3.5. Sample preparation

A 25-μl volume of plasma was pipetted into a 1-ml glass stoppered centrifuge test tube, 5 μl of the internal standard solution and 25 μl of borate buffer (pH 9.0) and 400 μl of dichloromethane were added. The sample was shaken for 5 min, centrifuged at 1930 × g for 10 min. The organic layer was transferred into the separate test tube and evaporated to dryness under a gentle stream of nitrogen. The residue was redissolved in 40 μl methanol. A 5 μl aliquot of the solution was injected onto the HPLC column.

3.6. Preparation of the standard curves

Calibration standards were prepared by adding 5 μl of the appropriate working standard and 5 μl of the internal standard solution and, to 25 μl of plasma. Five calibration concentrations of BCH were used for the standard curve in plasma – 0.01, 1, 5, 10 and 20 μg/ml.
Concentration of BCH were calculated from the linear regression equation of the calibration curve constructed by plotting the peak area ratio (y) of BCH and the internal standard versus the concentration of BCH.

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