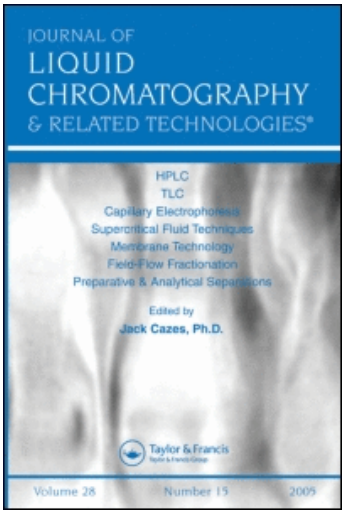

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Determination of Cinnarizine in Whole Blood and Plasma by Reversed Phase HFLC and its Application to a Pharmacokinetic Study

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DETERMINATION OF CINNARIZINE IN WHOLE BLOOD AND PLASMA BY REVERSED PHASE HPLC AND ITS APPLICATION TO A PHARMACOKINETIC STUDY

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ABSTRACT

Cinnarizine is determined in whole blood and plasma by reversed phase HPLC on a RP-18 stationary phase. The one-step extraction is performed with a chloroform/hexane (2/3) mixture. A high recovery of 91% and a detection limit of 2 ng/ml are obtained as well as a good precision. The internal standard is meclozine. Pharmacokinetic parameters found are in accordance with data cited in literature.

INTRODUCTION

Cinnarizine, an antihistamine of the diethylenediamine type is widely used in the treatment of cerebral and peripheral vascular insufficiency (1). The plasmalevels in man are situated between 0 and 200 ng/ml. To determine cinnarizine in plasma a sensitive and precise analysis procedure is required.

The methods for the analysis of cinnarizine described in the literature (2-6) exhibit several disadvantages. Dell and Fiedler (2) detected only a metabolite of cinnarizine, benzhydrol, in the urine of rabbits and dogs. The determination consisted of a TLC separation and a U.V. photometry. The determination of cinnarizine itself was not attempted. Morrison et al. (3) described a

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GC method with a low recovery of about 60% and a quite high detection limit of 10 ng/ml. The GC procedure of Woestenborghs et al. (4) is characterized by a high recovery and a low detection limit, but a disadvantage is the time consuming extraction consisting of three successive steps. For the HPLC method of Hundt et al. (5) several criticisms are possible : the recovery of 67% is low, the three-step extraction is tedious, the detection was done at 285 nm while the maximal absorption is situated at 255 nm which means a loss of sensitivity with a factor 6. In the HPLC method of Nitsche et al. (6) a pre-column was used, which is profitable since it extends the life expectancy of the separation column. A disadvantage is the working temperature of 60°C which complicates experimental chromatographic conditions. In addition the selected internal standard has no structural similarity with cinnarizine in contrast with what is desirable for such an analysis. Therefore, it was desirable and necessary to look for an alternative way of analysis of cinnarizine in plasma.

EXPERIMENTAL

Apparatus

Varian LC 5060 equipped with a standard Varian 254 nm U.V. detector and a manual Valvo loop injector (loop size 100 µl). Detection was performed at 0.01 A.U.F.S.

The column was Ultrasphere RP-18 from Altex, packed with 5 µm particles (l = 250 mm, i.d. = 4.6 mm). A pre-column packed with 10 µm Ultrasphere RP-18 (Altex) was also used (l = 30 mm, i.d. = 4.6 mm).

Integrations were performed with a Varian Vista CDS 401, the signal to noise ratio was 2, the WI (= initial peak width) was 10.

pH measurements were done with an Orion Ionalyser 601 and a combined glass electrode.

Extractions were carried out in 30 ml glass centrifuge tubes, sealed with a PTFE-coated screw stop; a mechanical reci-

procating shaker (Orlando Valentini), a heating block Sillitherm® (Pierce), 5 ml glass tubes and high-purity nitrogen were also used.

Chromatography

The mobile phase consisted of a mixture of 9 parts of methanol and 1 part of phosphate buffer (pH = 7.0 and ionic strength 0.1). The eluents were always filtered through a 0.45 µm Millipore filter. The flow rate was 1.5 ml/min. All experiments were carried out at room temperature, a back-pressure of ca. 220 atm was obtained.

Reagents

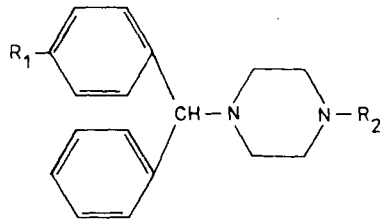
The chemical structure of cinnarizine and potential internal standards are given in Fig. 1. Cinnirazine and clocinizinehydrochloride were obtained from Janssen Pharmaceutica (Beerse, Belgium), buclizinehydrochloride, meclozinehydrochloride and chlorcyclizine were obtained from U.C.B. (Braine l'Alleud, Belgium). Methanol was HPLC-grade and sodiumphosphate, chloroform and hexane were analytical grade from Merck (Darmstadt, G.F.R.).

Stock Solutions

A cinnarizine stock solution was prepared by dissolving 80 mg of cinnarizine in 100 ml of methanol, 1 ml of this solution was then diluted to 500 ml with 0.1 N hydrochloric acid (concentration of the stock solution = 160 ng/100 µl). The stock solution of clocinizinehydrochloride was prepared as described for cinnarazine. The stock solution of meclozinehydrochloride was 4 mg in 100 ml of 0.1 N hydrochloric acid.

Determination of Extraction Recovery

a) from buffered aqueous solutions : 10 ml of the analyte's solution in phosphate buffer pH = 3.0 (1 mg/100 ml for cinnarizine and clocinizine, 35 mg/100 ml for meclozine) was extracted for



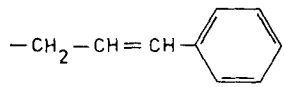
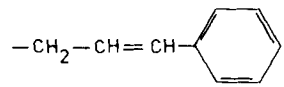
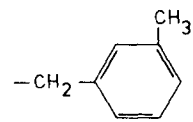
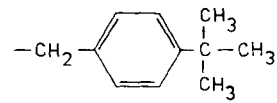
Name	R_1	R_2
Cinnarizine	H	$-CH_2-CH=CH-$ 
Clocinizine	Cl	$-CH_2-CH=CH-$ 
Chlorcyclizine	Cl	$-CH_3$
Meclozine	Cl	
Buclizine	Cl	

Figure 1

Structure of diethylenediamine antihistamines.

30 min with 10 ml of the organic phase. After phase separation, the amount of drug in the aqueous phase was determined by U.V. photometry

b) from biological fluids : to 4 ml of plasma or whole blood, transferred to a glass centrifuge tube, was added 250 µl of cinnarizine or clocinizine stock solution. For meclozine, 500 µl of the stock solution was added. The tube was then vortexed and 10 ml of phosphate buffer (pH = 3.0 and ionic strength 0.4) and 5 ml of organic phase were added. The tubes were sealed and shaken for 30 min on a mechanical shaker at the rate of 90 strokes/min. The tubes were centrifuged for 30 min at 2500 rpm. Three ml of the organic phase was transferred to a 5 ml glass tube, the solvent was evaporated at 40°C under a gentle stream of nitrogen. The residue was redissolved in 200 µl of methanol and 100 µl of this solution were injected in the HPLC system for analysis.

Pharmacokinetic Study

- 10 ml of blood was taken from six healthy volunteers after 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8 and 12 hours following administration. Blood was collected in heparinized syringes (Sarstedt Monovette®) which were immediately centrifuged, the plasma was separated from the blood cells and was frozen at -20°C until analysis
- calibration : to 4 ml of plasma was added 0.5 ml of internal standard solution and 0, 10, 50, 100, 200, 400 or 500 µl of cinnarizine stock solution. The analysis was then carried out as described under "extraction recovery from biological fluids", starting from : "The tube was then vortexed ...". Chloroform/hexane (2/3) was taken as the organic phase
- analysis of samples : to 4 ml of plasma was added 0.5 ml of internal standard solution. The analysis was then carried out as described under "extraction recovery from biological fluids", starting from : "The tube was then vortexed ...". Chloroform/hexane (2/3) was taken as the organic phase.

Determination of Cinnarizine in Single Capsules

Ten capsules were chosen at random, each capsule was opened and its content was transferred quantitatively to a centrifuge tube. 20 ml of methanol was added to the centrifuge tube which was then sealed, ultrasonicated for 5 min and shaken for 15 min on the mechanical shaker, the tube was centrifuged for 15 min at 2000 rpm, and the clear supernatant liquid was transferred to a 100 ml flask. The extraction was repeated twice with 20 ml fractions of methanol. The combined extracts were diluted to 100 ml with methanol and 1 ml of this solution was then diluted to 50 ml with methanol. 100 µl of this final solution was then injected in the HPLC system for analysis. Calibration was performed with cinnarizine standards which contained 0.5, 1.0, 1.5 and 2.0 mg of the solute in 100 ml of methanol.

RESULTS AND DISCUSSION

Selection of the Chromatographic System

Cinnarizine, which is a lipophilic drug, is nearly insoluble in water and is strongly bound to the RP-18 stationary phase. In order to elute it, a mobile phase with a high methanol content is required. The pH of the mobile phase is important in view of the separation of cinnarizine from matrix constituents. The mixture methanol/phophate buffer pH = 7 (9/1) was revealed to be the most successful of the systems investigated since it permitted the baseline resolution of cinnarizine from endogenic plasma or blood compounds. Furthermore, a detection limit of 0.5 ng was obtained for this system at 0.005 absorbance units full scale, a signal to noise ratio of 2 and a WI value of 10. These latter parameters fix the slope sensitivity of the integrator. Eight replicate injections of 1 ng of cinnarizine yielded a relative standard deviation of 10.3%. Finally, the use of such a high methanol content permitted a flow rate of 1.5 ml/min even with the 5 µm stationary phase. This shortened the analysis time but had no negative effect on column life due to high back-pressures. The performance

TABLE 1
Retention Times of Diethylenediamine Antihistamines on Ultrasphere RP-18 (l = 25 cm, dp = 5 μm). Mobile Phase : Methanol/Phosphate Buffer pH = 7 (90/10). Flow : 1.5 ml/min.

Name	Retention time (min)
Chlorcyclizine	5.8
Cinnarizine	6.8
Clocinizine	8.9
Meclozine	10.7
Buclizine	17.6

of the column was stable for several months which corresponds to the injection of several hundreds of extracts of biological fluids. The pre-column was changed after ca. 100 injections of extracts. It should be emphasized that this determination required the use of a 5 μm stationary phase in order to get the detection limit down to the required low level and to obtain satisfactory separations.

Selections of the Internal Standard

The internal standard was chosen among the diethylenediamine antihistamines shown in Fig. 1. Their structures, and consequently their U.V. absorption and extraction characteristics are very close to cinnarizine's properties. As can be seen in Table 1, chlorcyclizine elutes before cinnarizine but is incompletely resolved from matrix compounds. On the other hand, buclizine's retention is too high. Two possibilities remain : meclozine and clocinizine, of which clocinizine should be preferred in view of its greater resemblance with cinnarizine.

Selection of the Extraction Scheme

Many papers published in the literature describe the extraction of basic drugs from biological fluids. Such extractions are performed mostly from a basic medium and with various organic solvents. The extraction scheme consists very often of further

purification steps, e.g. a back- and re-extraction. Some procedures for cinnarizine cited in the literature (4-5) use such a methodology. In our laboratory, a standardized extraction procedure for basic drugs has been developped (8). It consists of an ion-pair extraction with n-octylsulphate at pH = 3.0 or HDEHP (=di-(2-ethylhexyl)phosphoric acid) at pH = 5.5 with chloroform as the solvent. It yields pure extracts and also high recoveries. The method is especially interesting for hydrophilic drugs which are very difficult to extract by classical means. Cinnarizine is lipophilic and is extracted quite well to chloroform (3) or tetrachlorocarbon (4) even from acidic solutions. Preliminary experiments carried out on aqueous buffered solutions (pH = 3.0) showed that upon addition of 2.5×10^{-2} M octylsulphate a recovery of 98% to chloroform was obtained, which is equal to the yield obtained with chloroform alone. As both extractions are equally good, the simplest one was chosen for routine application.

As stated by Hundt (5) the pH has no distinct influence on the extraction recovery to chloroform. Experiments were carried out at pH = 3 since at a higher pH higher blanks were obtained. The efficiency of the extraction to chloroform and hexane is given in Table 2. In order to facilitate manipulations (e.g. the transfer of a known amount of organic phase) during the extraction of drugs from blood or plasma, a supernatant organic phase is preferable. A chloroform/hexane mixture (2/3), which has a density less than one, was used by our laboratory for the determination of papaverine in blood (7). The recoveries obtained with this mixture for cinnarizine and the internal standards are nearly quantitative and are also given in Table 2.

Extractions carried out on whole blood and plasma indicate that the chloroform/hexane mixture is a much better extractant than chloroform alone (Table 3a). As shown in Fig. 2, the extract with chloroform/hexane is also purer than with chloroform. Furthermore, it was observed that in most plasma samples analysed, a peak was obtained which interfered with clocinizine. Therefore meclozine was preferred as the internal standard since no interference was observed and because of its higher extraction yield for plasma (Table 3b).

TABLE 2

Extraction Efficiency (%) from 10 ml of Aqueous Buffer Solution pH = 3.0 to 10 ml of Organic phase. N = 3

Substance	Organic Phase		
	Chloroform	Hexane	Chloroform/Hexane (2/3)
Cinnarizine	98.5 ± 0.5	67.0 ± 0.8	96.7 ± 2.1
Clocinizine	98.4 ± 0.1	94.7 ± 0.6	98.0 ± 0.2
Meclozine	94.2 ± 0.2	97.9 ± 0.6	99.6 ± 0.8

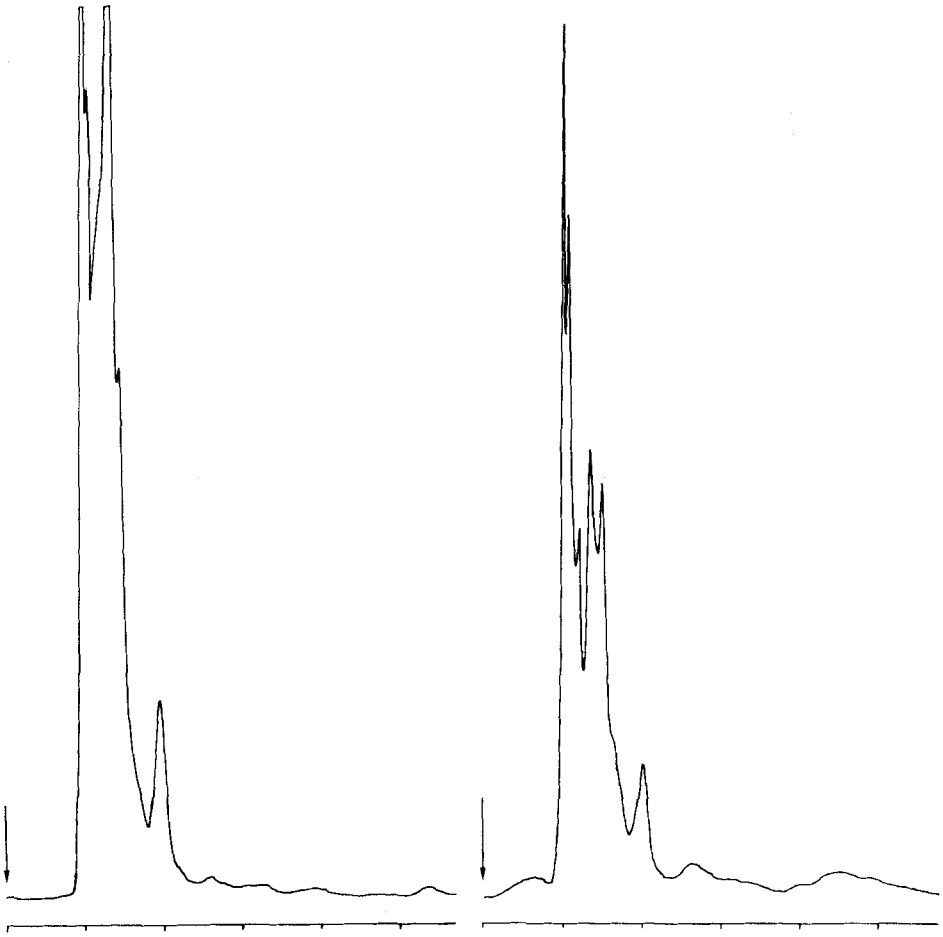


Figure 2

Blank extracts from whole blood.

A : with chloroform

B : with chloroform/hexane (2/3)

Chromatographic conditions as in Table 1.

TABLE 3
Extraction Efficiency (%) from 4 ml of Whole Blood or Plasma.
N = 3, *N = 10

	Chloroform	Chloroform/Hexane (2/3)
a) from whole blood :		
Cinnarizine	33.0 ± 1.8	82.0 ± 1.5
Clocinizine	36.3 ± 1.0	85.5 ± 0.9
b) from plasma :		
Cinnarizine	55.2 ± 2.1	90.9 ± 3.3*
Clocinizine	31.8 ± 0.9	60.3 ± 3.5
Meclozine	36.5 ± 1.1	87.0 ± 2.9*

Quality of the Analysis Scheme

As given above, the extraction efficiencies are 91% for cinnarizine and 87% for the internal standard. Calibration curves were constructed by spiking increasing volumes, ranging from 10 to 500 µl, of the cinnarizine stock solutions (160 ng/100 µl) and 500 µl of the meclozine stock solution (4 µg/100 µl) to 4 ml of blank plasma. Calibration curves were constructed by plotting the ratio of the peak areas of cinnarizine to meclozine versus the concentration of cinnarizine (4 - 200 ng/ml plasma). For six curves constructed on different days the following equation was obtained :

$$\text{relative peak area} = 0.0059\ (\pm 0.0005) \times (\text{concentration}) - 0.0308\ (\pm 0.0446)\ (\text{ng/ml plasma} = \text{conc.})$$

the mean correlation coefficient was 0.9991

This indicates that the slope of the calibration curve has a between-day variation of about 8%. All determinations carried out on a single day were always referred to a calibration curve which was also constructed on the day of analysis.

The limit of detection is 2 ng/ml plasma. The precision of the method was determined at two concentration levels, e.g. 20 and 200 ng cinnarizine/ml plasma. The within-day precision is

7.5% at the 20 ng/ml level and 2.9% at the 200 ng/ml level. The between-day precision is 7.7% at the 20 ng/ml level and 5.3% at the 200 ng/ml level. In both cases the number of replicates was 6.

Pharmacokinetic Study

A number of pharmacokinetic parameters were determined for six healthy subjects following oral administration of 75 mg of

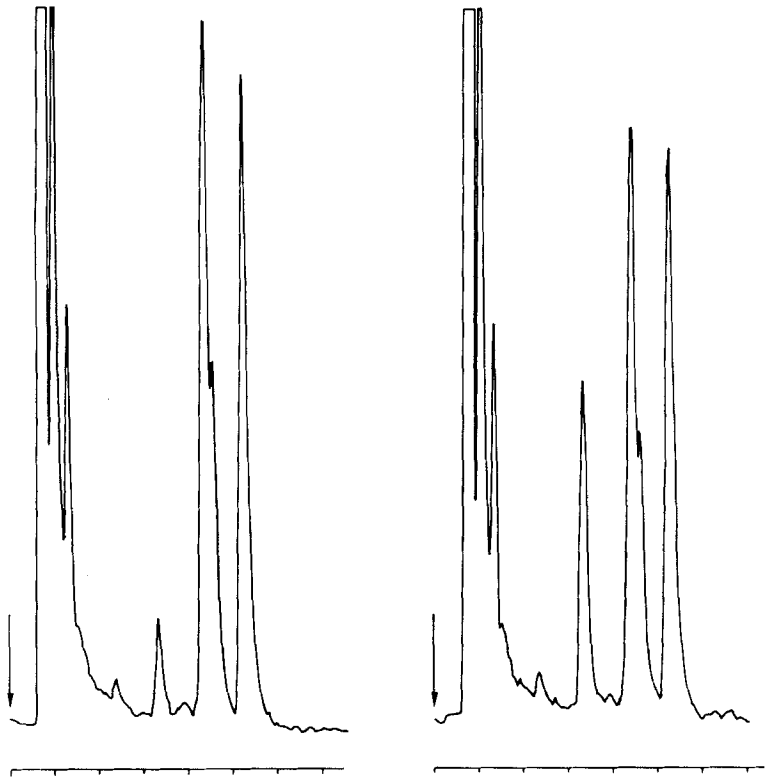


Figure 3

Determination of cinnarizine in plasma samples.
A : 1.5 h after administration
B : 2 h after administration
Chromatographic conditions as in Table 1.

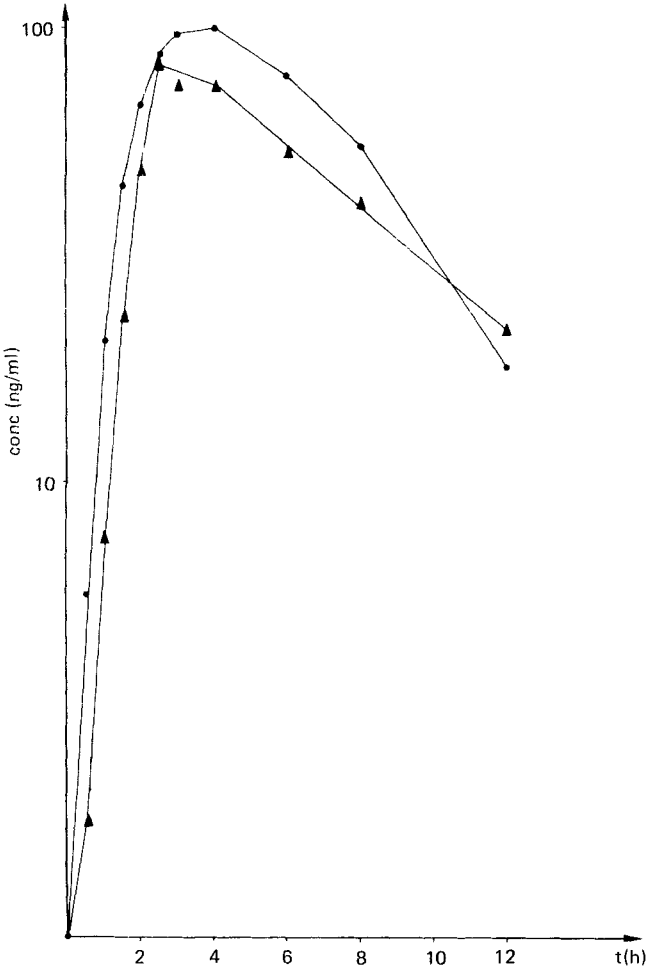


Figure 4

Mean cinnarizine concentration in plasma as a function of time following oral administration of 75 mg of cinnarizine

● : capsules A

▲ : capsules B

Chromatographic conditions as in Table 1.

TABLE 4

Pharmacokinetic parameters : C_{max} = peak concentration, T_{max} = time of peak concentration, T 1/2 = half-life, A.U.C. = area under the curve

	C_{max} (ng/ml)	T_{max} (h)	T 1/2 (h)	A.U.C. (ng x h/ml)
75 mg Cinnarizine				
Capsules A (N=6)	122± 75	3.3±1.6	2.79±1.04	855±431
Capsules B (N=6)	110±133	4.1±2.0	3.24±2.13	637±621
Capsules 75 mg (N=12) (3)	230±130	2.6±1.0	3.43±0.83	1277±440
Tablets 75 mg (N=12) (3)	160±130	3.4±1.2	3.04±1.54	925±603
50 mg Cinnarizine (5)				
Tablets A (N=6)	76± 35	2.3±0.4	4.4 ±1.0	583±180
Tablets B (N=6)	89± 42	2.4±1.1	5.3 ±1.6	721±268

cinnarizine. Two brands of capsules, with similar composition, were compared. Prior to the study the content of single capsules was tested for both brands. The theoretical content being 75 mg, brand A contained 73.9 ± 1.7 mg of cinnarizine, brand B : 73.5 ± 2.0 mg (N=10).

The plasmalevels were determined in samples taken at regular intervals between 0 and 12 hours following oral administration of 75 mg of cinnarizine formulated as capsules. In Figure 3, chromatograms are shown from spiked as well as real plasma samples. The mean plasma concentrations are plotted versus time in Figure 4 for both brands. In Table 4 an overview of the pharmacokinetic parameters obtained from the experimental data are given together with the data from the literature (3) (5). A.U.C. (= area under the curve) values, which are a measure of the bioavailability of a drug, were calculated with the "Estrip" program (9). The data indicate that there is a high biovariability. This is pointed out by our results as well as by the

results found in the literature. The two brands tested out by us do not differ significantly. Considering our data and those found in the literature, one may conclude that after oral absorption of 75 mg of cinnarizine the maximal plasmaconcentration is attained after ca. 3.3 h and the half-life is ca. 3.2 h. The values of the maximal concentration (170 ng/ml) and A.U.C. (980 ng x h/ml) are less certain because of the larger between-brand differences which are observed. Comparison of the data for 75 and 50 mg show that at 50 mg the peak maximum is obtained in a shorter time (ca. 2.3 h) and that the elimination is somewhat slower (t 1/2 = half-life = ca. 4.8 h).

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