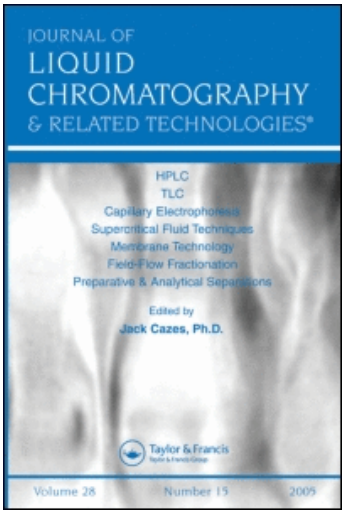

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AUTOMATED DETERMINATION OF AN ANGIOTENSIN II RECEPTOR ANTAGONIST, CGP 48 933, IN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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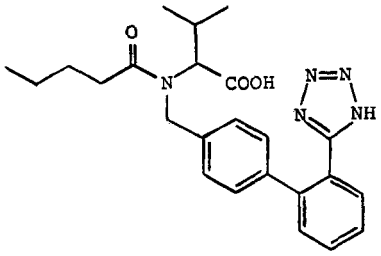
ABSTRACT

A fully automated high-performance liquid chromatography method with fluorimetric detection is described for the determination of CGP 48 933 in human plasma. Liquid-solid extraction was performed automatically on C8 reversed phase column using the Gilson ASPEC system. The on-line chromatography was performed on a ODS Hypersil C18 5 μm column. The mobile phase, acetonitrile- pH 2.8 phosphate buffer (50:50, v/v) was used at a flow rate of 1.3 ml/min. The fluorimetric excitation and emission wavelengths were set at 265 and 378 nm, respectively. The limit of quantitation of CGP 48 933 was 11.5 nmol per litre of plasma.

INTRODUCTION

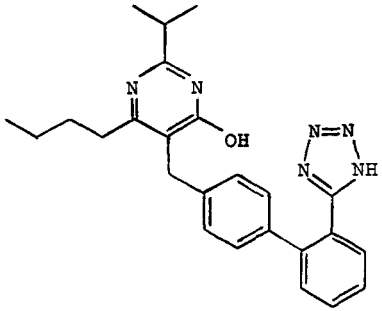
CGP 48 933 is a new class of antihypertensive compound (Figure 1) which acts through blockade of the angiotensin II receptors. The renin-angiotensin system which plays an important role in the regulation of blood pressure, may be blocked either by angiotensin converting enzyme inhibitors or at the angiotensin II receptors.

To investigate the pharmacokinetics of CGP 48 933 in humans, a high performance liquid chromatography method was developed using a structural analog as internal standard (Figure 1). Previously, a HPLC method was described for the determination of another compound with similar activity (1).



CGP 48 933

Mol. wt. = 435.52



CGP 48 791
Internal standard

Mol. wt. = 428.55

Figure 1 : Chemical structures.

MATERIALS

CGP 48 933 and the internal standard were supplied by Ciba-Geigy (Basle, Switzerland).

The reagents were all of analytical grade. Acetonitrile for HPLC, methyl alcohol for UV and potassium dihydrogenphosphate were purchased from Carlo Erba France. 0.1 N hydrochloric acid, 0.1 N sodium hydroxide and 85 % phosphoric acid were from Merck (Darmstadt, Germany). Water was purified with a Millipore Milli-Q system (Millipore, France).

Disposable extraction columns (DECs) of 1 ml capacity containing 100 mg C8 reversed phase(Bond-Elut, Analytichem International, Varian, France) were used for liquid-solid extraction.

Apparatus

The chromatographic equipment was as follows :

. A fully automated analytical system (ASPEC, Gilson, Villiers-le-Bel, France), based on automated liquid-solid extraction and injection combined with HPLC.

- A solvent delivery system, pump model 302 associated with a manometric module model 802C (Gilson).
- A fluorimetric detector Hitachi, model 1050 (Merck,France). The excitation and emission wavelengths were set at 265 and 378 nm, respectively.
- A data pool workstation NEC 810 (Waters, Millipore, France) which performed integration, recording and storage of the data.

Chromatographic conditions

A pre-packed column ODS Hypersil C18 (150 mm x 4.6 mm I.D., 5 µm particle size, Hewlett-Packard, France) was used for the separation. To protect this column, a guard column Supelguard LC-8, 20 mm x 4.6 mm (Supelco, France) was installed.

The buffer of the mobile phase was prepared by dissolving 1.36 g of potassium dihydrogenphosphate in one litre of distilled water. The final pH 2.8 was adjusted with thirteen drops of 85 % phosphoric acid. The mobile phase (pH 2.8 phosphate buffer-acetonitrile (50:50, v/v) was used at a flow rate of 1.3 ml.

Standard and validation solutions

Standard and validation solutions were obtained by dissolving 2.29 µmol (1 mg) of CGP 48 933 in 50 ml of methanol and by successive dilutions of the master solution in methanol. The plasma standard concentrations of CGP 48 933 ranged from 11.5 nmol/l to 4600 nmol/l.

2.33 µmol (1 mg) of internal standard (I.S.) was dissolved in 50 ml methanol.

METHODS

Sample extraction

100 mg C8 DECs were placed on the rack of the ASPEC. An aliquot of the I.S. solution was introduced into a 5 ml polypropylene tube and evaporated to dryness. After addition and mixing of 1 ml of plasma, the tube was placed on the rack of the ASPEC system.

300 µl of the eluate were dispensed through the 50 µl injection loop.

Each plasma sample was prepared separately during the chromatography of the previous sample.

CGP 48 933 and I.S. were found to be partly adsorbed on the needle of ASPEC. A preliminary washing of the needle with methanol and 0.1 N NaOH before each sample preparation was necessary to avoid cross-contamination.

All the following operations on the samples were automatically performed :

Successive steps	Liquid dispensed on the DEC	Dispensing flow rate µl/sec (ASPEC code)	Pressurising air volume (µl)
DEC conditionning	1. Methanol 2 ml 2. 0.1 N HCl 2 ml	100 (code 5)	50
Sample distribution	1 ml 0.1 N HCl was added to 1 ml plasma and the 2 ml were dispensed on the DEC	6 (code 1)	1000
Washing	1. 1 ml KH_2PO_4 pH2.8 2. 0.5 ml KH_2PO_4/ CH3CN (70/30,v/v)	50 (code 4)	1000
Elution	2 ml of mobile phase (50:50, v/v)	50 (code 4)	1000

Calibration curve

Calibration samples were prepared by adding aliquots of various methanolic solutions of the compound to plasma. The calibration curves were established from the peak height ratio compound/I.S. plotted versus the concentrations of CGP 48 933 in the samples. Their equations were calculated by the least-squares method using weighted linear regression with a weighting factor of $1/(\text{concentration})^2$.

RESULTS

As shown in Figure 2, CGP 48 933 and internal standard were well separated from human plasma components. No endogenous peaks interfered with the detection of CGP 48 933 and I.S. and under the chromatographic conditions the retention times of the compounds were 5 and 8 min, respectively.

An example of a calibration graph is given in Figure 3. Plasma samples containing different concentrations of CGP 48 933 were repeatedly analysed either on the same day in six replicate (within-day precision) or on five consecutive days (between-day precision).The results obtained with the procedure described are given in Tables 1 and 2, where the recovery (%) was calculated as the given concentration/found concentration multiplied by 100.

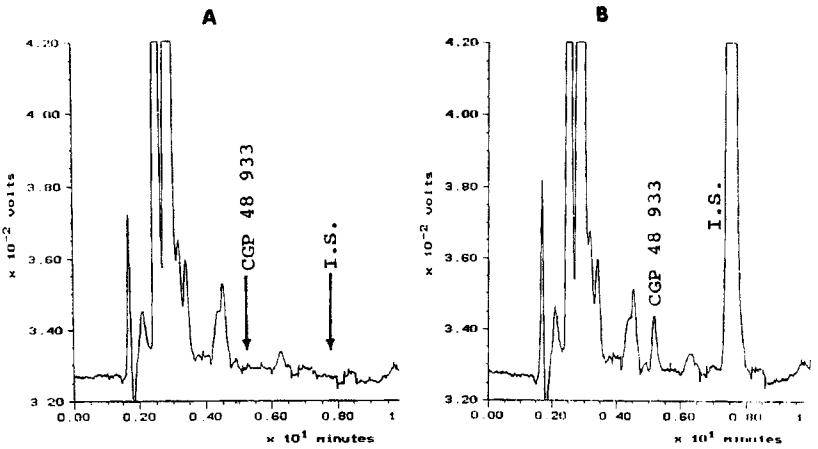


Figure 2 : Example of chromatograms obtained from an extract of 1 ml human plasma.
(A) : 1 ml drug-free plasma.
(B) : 1 ml plasma spiked with 23 pmol (10 ng) of CGP 48 933 and 1167 pmol (500 ng) of I.S.

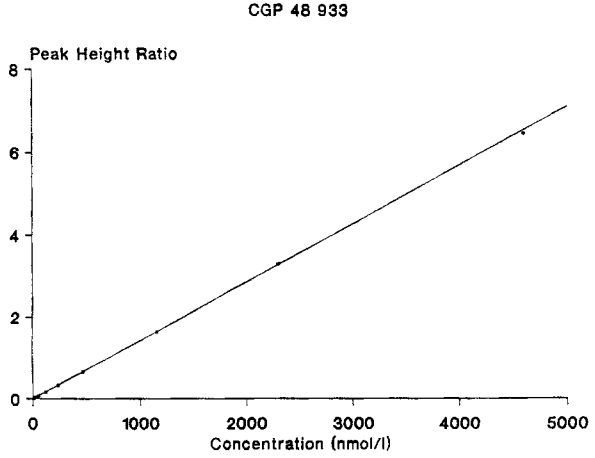


Figure 3 : An example of calibration curve of CGP 48 933.
Slope : 0.00142.
Intercept : - 0.00174.
Correlation coefficient : 0.999.

TABLE 1

Within-day reproducibility and accuracy of the determination of CGP 48 933 in human plasma.

Given (nmol/l)	11.5	23.0	920	3450
Recovery (%)	109	99.8	96.9	101
	98.1	98.0	98.3	97.8
	98.1	95.5	97.9	98.2
	101	108	97.6	98.5
	96.9	91.3	96.8	98.6
	93.2	95.5	98.9	97.1
Mean (%)	99.4	98.0	97.7	98.5
CV (%)	5.4	5.8	0.8	1.3
SD	5.3	5.7	0.8	1.3

TABLE 2

Day-to-day reproducibility and accuracy of the determination of CGP 48 933 in human plasma.

Given (nmol/l)	11.5	23.0	57.5	920	3450
Day of analysis	Recovery (%)				
2	102	103	100	98.5	96.2
3	100	96.2	93.6	95.3	97.8
4	104	92.9	97.6	98.0	102
5	100	99.1	96.6	98.6	101
8	109	99.5	97.2	99.2	93.2
Mean (%)	103	98.1	97.0	97.9	98.0
CV (%)	3.6	3.9	2.4	1.6	3.7
SD	3.7	3.8	2.3	1.5	3.6

The limit of quantitation (coefficient of variation < 10 %) was calculated from the results in Table 1 and was 11.5 nmol/l of plasma.

The standard and validation solutions of CGP 48 933 and the I.S. solution were stable for at least three weeks at +4°C. This was determined by injecting daily aliquots of internal standard and compound solutions : as regards to the peak height, the results obtained over three weeks were compared with those obtained on the first day.

For a plasma sample spiked with a given amount of CGP 48 933 and I.S., a similar peak height was observed for the two compounds with extraction performed either immediately after preparation or 12 hours later. This indicated that there was no degradation of CGP 48 933 and the I.S. in the diluted plasma samples left at room temperature on the rack of the ASPEC system for several hours.

DISCUSSION

Various DEC's sorbent were tested. The extraction recoveries and the separation of the compounds from the plasma components were explored.

With 100 mg C2 DECs, the extraction recoveries were variable depending on the batch of the DECs. With 100 mg C8 or C18 DECs, the extraction recoveries were found more reproducible from batch to batch and were around 90 %.

These recoveries were similar to those of others studies (2, 3) which reported extraction recoveries from DECs around 90 %.

It appeared that high recoveries were required to obtain a good reproducibility over the time and the various batches of DECs.

A previous manual method for the plasma determination of CGP 48 933 using DECs had been developed in our laboratory. The results obtained with the two HPLC methods were compared. The limit of quantitation with a coefficient of variation inferior to 10 % was improved by 2 with the ASPEC system and the precision and accuracy were slightly better as shown in Table 3.

Given (nmol/l)	11.5	23.0	23.0
Day of analysis	Recovery (%)		
	ASPEC System		Manual
2	102	103	95.1
3	100	96.2	105
4	104	92.9	104
5	100	99.1	98.5
8	109	99.5	93.7
Mean (%)	103	98.1	99.7
CV (%)	3.6	3.9	5.6
SD	3.7	3.8	5.6

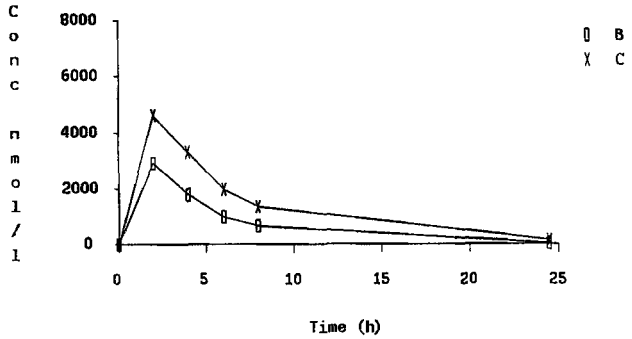


Figure 4 : Mean plasma concentration-time profiles after oral administration to six volunteers of either 40 mg (B) or 80 mg (C) of CGP 48 933.

APPLICATION

This method was applied to plasma samples from volunteers given a single oral doses of CGP 48 933. The mean plasma concentration-time profiles are shown in Figure 4. At sampling-time 24 h, the lowest concentration observed was three fold the limit of quantitation. So the sensitivity of this method was suitable for pharmacokinetic investigation.

CONCLUSIONS

An automated HPLC method has been developed and validated to quantify CGP 48 933 in plasma samples. The sensitivity of this method was improved compared to a manual method and was suitable to study the phamacokinetics of the compound.

REFERENCES

1. R. H.-Y. Chiou, M.-W. Lo, J. Chromatogr., 581: 165-170 (1992).

2. M.C. Rouan, J. Campestrini, V. Le Clanche, J.B. Lecaillon, J. Godbillon, J. Chromatogr., 573: 65-68 (1992).

3. G. Hotter, G. Gomez, I. Ramis, G. Bioque, J. Rosello-Catafau, E. Gelpi, J. Chromatogr., 607: 239-243 (1992).

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