

Short Communication

High-performance liquid chromatographic method for the assay of TA-3090 and its pharmacokinetic parameters in healthy volunteers*

MARC LEFEBVRE,† D. GARCEAU, J. SPENARD, J. M. HOULE, D. MAJOR and G. CAILLE

Département de Pharmacologie, Faculté de Médecine, Université de Montréal, C.P. 6128 Succ. A, Montréal, Québec, Canada, H3C 3J7

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Introduction

TA-3090, a chloro-derivative of Diltiazem, is a new calcium channel blocker from the benzothiazepine family (Fig. 1). This new drug exhibits interesting properties: selective cerebral and coronary vasodilating action, hypotensive effect, and a weak inotropic effect. The calcium antagonist action of TA-3090 is 2 to 10 times as potent as that of Diltiazem in vascular smooth muscles and also has a longer lasting effect [1–4]. However, the pharmacokinetic handling of TA-3090 is still very restricted in man.

No method has been published yet for the analysis of TA-3090 in biological fluid. In the present article, a high-performance liquid chromatographic (HPLC) method is described for the simultaneous determination of TA-3090 and its two principal metabolites, TA-DAD and TA-MA (the desacetyl and *N*-desmethyl derivative,

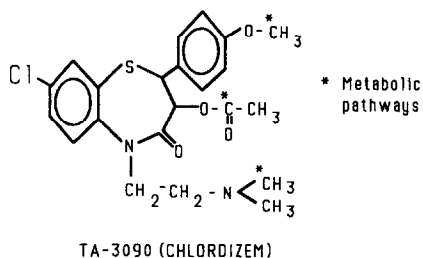


Figure 1
Structure of TA-3090.

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† To whom correspondence should be addressed.

respectively) in plasma. The method is established to be specific, sensitive and reproducible. This method has been applied to the measurement of TA-3090, TA-MA and TA-DAD in healthy volunteers receiving single doses of TA-3090 varying from 20 to 120 mg.

Materials and Methods

Stability

One important point to note concerns TA-3090, TA-MA and TA-DAD. Degradation can occur after a few hours at room temperature where TA-DAD is produced by the degradation of TA-3090; on the other hand, TA-MA is also unstable and its degradation product is TA-M2 (the desacetyl, *N*-desmethyl derivative).

For the standardization of this study, the stability of TA-3090, TA-MA and TA-DAD has been evaluated in solution (HCl 0.01 N) at two temperatures, 4 and 20°C. This study indicated that they were stable at 4°C for at least 1 month, but that degradation of 10, 12 and 4%, respectively, were noted after 2 weeks at room temperature and 18, 26 and 6% after 1 month.

Extraction

To 2 ml of plasma contained in a culture tube, 100 μl of 1 mg l⁻¹ of loxapine was added as internal standard. The sample was vortexed for 10 s and extracted with 6 ml of diethylether and 3 ml of a phosphate buffer adjusted to pH 7.5. After agitation on a reciprocating shaker for 10 min and centrifugation at 2500 rpm for another 10 min, the organic phase was then transferred to a second culture tube and back-extracted in 150 μl of 0.05 N sulphuric acid. After centrifugation for 4 min at 2500 rpm, the acidic phase (60 μl) was injected into the HPLC.

Apparatus

The assays were performed on a high-performance liquid chromatograph (Waters model 501, solvent delivery system, Millipore Corp., Massachusetts, USA) equipped with a UV detector (Waters model 441, absorbance detector, Millipore Corp., Massachusetts, USA) set at 229 nm and at 0.005 absorbance units full scale (AUFS). The 15 \times 0.46 cm reversed-phase C-18 column was packed with 10- μm Spherisorb ODS-1 (Chromatographic Sciences Company, Montreal, Quebec, Canada) and eluted with acetonitrile-phosphate buffer pH 3 (35:65, v/v) with the addition of 4.2 $\mu\text{mol ml}^{-1}$ of dibutylamine. The mobile phase was at a flow rate of 1.8 ml min⁻¹ and no interferences from endogenous substances were found. Under these chromatographic conditions, good separation and identification of TA-DAD, the internal standard (loxapine), TA-MA and TA-3090 were obtained. Following this order of detection, retention times ranged from 6.3 up to 11 min, with a minimum of 1 min separating each peak (Fig. 2). The other metabolites (TA-M2, TA-M4 and TA-M6), being more polar, did not interfere with the conditions used here for TA-3090, TA-MA and TA-DAD.

Results and Discussion

Calibration curves

Standard samples were prepared by spiking human plasma with increasing concentrations (2.5, 5, 15, 25, 50 and 75 ng ml⁻¹) of TA-3090, TA-MA and TA-DAD.

- RETENTION TIMES:

I TA-DAD	6:3 min.
II LOXAPINE	7:3 min.
III TA-MA	8:5 min.
IV TA-3090	11:0 min.

- CHROMATOGRAMS

Figure 2
Typical chromatogram of TA-3090, TA-MA and TA-DAD of a plasma extract.

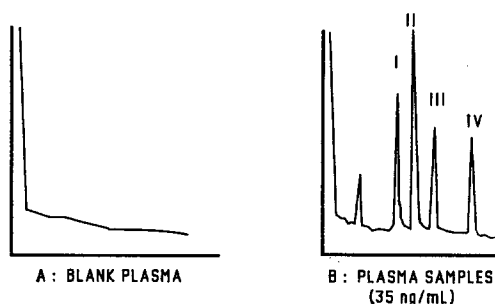


Table 1
Calibration curves of TA-3090, TA-MA and TA-DAD assay in plasma

Conc. (ng ml ⁻¹)	Peak height ratios (\pm SD)		
	TA-3090 $\times 10^{-2}$	TA-MA $\times 10^{-2}$	TA-DAD $\times 10^{-2}$
2.5	2.7 \pm 0.3	3.2 \pm 0.6	6.4 \pm 0.5
5.0	5.9 \pm 0.1	6.2 \pm 0.3	10.7 \pm 0.4
15.0	16.1 \pm 1.1	19.2 \pm 1.9	31.4 \pm 1.7
25.0	27.3 \pm 1.4	33.2 \pm 2.4	54.4 \pm 2.9
50.0	58.0 \pm 3.3	67.2 \pm 4.7	113.8 \pm 4.9
75.0	84.4 \pm 4.0	89.3 \pm 4.4	165.1 \pm 9.9
Calibration data	TA-3090 : $y = 0.0114x - 0.0008$		$r^2 = 0.9965$
	TA-MA : $y = 0.0133x - 0.0018$		$r^2 = 0.9880$
	TA-DAD : $y = 0.0222x - 0.0051$		$r^2 = 0.9957$

Calibration curves were constructed by plotting the ratios of the peak height of these three substances to that of the internal standard against the representative concentrations on different times (Table 1). The squared linear regression coefficient (r^2) of TA-MA was lower than that for the two others, but this can be explained by its poorer stability; despite this fact, it is still very acceptable. Thus it can be concluded that these results are linear throughout the range studied for these three products.

The day-to-day reproducibility and the sensitivity data of the method are presented in Table 2. Peak height ratios were calculated for each concentration and the RSD was used to analyse these parameters. In fact, as 10% of variation was fixed as maximum value for the assay of TA-3090, TA-MA and TA-DAD, the sensitivity of the method was acceptable at 2.5 ng ml⁻¹, even if the limit of detection was 1.0 ng ml⁻¹. Then good day-to-day reproducibility of this method was obtained with concentrations of TA-3090, TA-MA and TA-DAD as low as 2.5 ng ml⁻¹.

Precision and accuracy

Results are presented respectively in Tables 3 and 4. Firstly, precision was evaluated by the extraction of plasma samples at three different concentrations (10, 25 and 50 ng ml⁻¹) and the concentrations found were calculated using the equation derived from the

Table 2
Sensitivity and day-to-day reproducibility of TA-3090, TA-MA and TA-DAD assay

Conc. (ng ml ⁻¹)	TA-3090 × 10 ⁻²	Peak height ratios (±SD)			TA-DAD × 10 ⁻²	RSD (%)
		RSD (%)	TA-MA × 10 ⁻²	RSD (%)		
2.5	2.7 ± 0.3	10.4	3.3 ± 0.2	6.5	6.2 ± 0.1	1.6
5.0	5.7 ± 0.5	9.9	6.6 ± 0.7	10.7	12.3 ± 0.2	1.7
10.0	10.9 ± 0.9	8.2	12.6 ± 0.8	6.7	23.5 ± 0.6	2.4
25.0	24.0 ± 0.3	1.3	30.9 ± 0.1	0.3	57.3 ± 2.3	4.1
50.0	57.5 ± 1.5	0.3	61.8 ± 1.3	2.1	109.5 ± 9.8	8.9

Sensitivity of assay = 2.5 ng ml⁻¹
Sensitivity of detection = 1.0 ng ml⁻¹

Table 3
Precision of TA-3090, TA-MA and TA-DAD assay

	Conc. (ng ml ⁻¹)	Conc. found (ng ml ⁻² ± SD)	Error (%)	RSD (%)
TA-3090	10	10.4 ± 0.5	3.5	4.9
	25	25.8 ± 1.8	3.0	7.1
	50	49.0 ± 2.6	2.0	5.2
TA-MA	10	9.8 ± 1.6	2.2	13.8
	25	24.4 ± 1.2	2.2	4.9
	50	49.7 ± 2.3	0.7	4.6
TA-DAD	10	10.2 ± 0.3	1.5	3.1
	25	23.2 ± 0.9	7.3	3.8
	50	47.7 ± 2.0	4.6	4.2

Table 4
Accuracy of TA-3090, TA-MA and TA-DAD assay

	Conc. (ng ml ⁻¹)	Conc. found (ng ml ⁻¹)	Extraction (%)
TA-3090	5	4.34	86.8
	50	43.52	87.0
	70	63.23	90.3
TA-MA	5	4.32	86.4
	50	42.38	84.8
	70	59.46	84.9
TA-DAD	5	4.44	88.8
	50	47.80	95.6
	70	65.38	93.4

calibration curves described before. The precision is a quality control to verify the reproducibility, within day, of the method which is also estimated by the RSD. All values were under 7.1% except for one value of TA-MA (13.8%). The poorer stability of this metabolite could be again the cause of this variation. In spite of this factor, the percentage of error representing the deviation from the nominal concentration is under 7.3% (Table 3). In regard to these results, good precision was confirmed with this method.

Secondly, accuracy (recovery) of the method concerning TA-3090, TA-MA and TA-DAD was determined by comparing the peak height ratios obtained by direct injection of standard solution in HCl with those obtained after the extraction procedure (loxapine was used as an external standard for the extracted samples). Three concentrations (5, 50 and 70 ng ml⁻¹) were assayed and the concentrations found represent the recovery obtained from the extraction of plasma samples (Table 4). Thus, the recoveries of TA-3090, TA-MA and TA-DAD all exceed 85% and confirm the accuracy of the extraction procedure.

Spiked samples

Finally, 90 samples of the following concentrations (4, 50 and 300 ng ml⁻¹), prepared in the USA, were assayed for TA-3090, TA-MA and TA-DAD. These unknown concentrations demonstrated RSD values lower than 8.0% and the amounts found correlated well with the real concentrations of TA-3090 and its metabolites (Table 5). This final step confirmed the validity of the method of analysis.

Table 5
Spiked plasma samples of TA-3090, TA-MA and TA-DAD assay

Conc. (ng ml ⁻¹)		Percentage found (% ± SD)	RSD (%)
4	TA-3090	103.3 ± 6.7	6.52
	TA-MA	110.5 ± 8.8	7.99
	TA-DAD	122.2 ± 8.3	6.76
50	TA-3090	94.0 ± 3.6	3.87
	TA-MA	104.6 ± 5.2	4.94
	TA-DAD	98.8 ± 4.4	4.47
300	TA-3090	98.6 ± 3.6	4.01
	TA-MA	111.6 ± 5.0	4.46
	TA-DAD	104.4 ± 4.4	4.24

Pharmacokinetic study

The assay procedure has been applied to the determination of the pharmacokinetics of TA-3090 in healthy volunteers: this single dose study was performed with increasing weekly doses (20, 40, 60, 80, 100 and 120 mg) and blood samples were taken at 0, 1, 2, 3, 4.5, 6, 12, 16, 24 and 30 h after each administration of the oral dose. Mean maximum plasma concentrations ($C_{\max} \pm \text{SEM}$) seemed to correlate well with all doses (9.0 ± 0.9 ng ml⁻¹ up to 117.4 ± 10.7 ng ml⁻¹) and the mean time to C_{\max} (T_{\max}) was around 2 h for all (Fig. 3).

The mean areas under the plasma concentration time curve ($\text{AUC} \pm \text{SEM}$) determined at infinity were linear throughout the range studied (120.8 ± 21.6 ng·h ml⁻¹ up to 1140.2 ± 71.3 ng·h ml⁻²) (Fig. 4).

Finally, the mean half-life time ($t_{1/2(\text{el})} \pm \text{SEM}$) was 10.2 ± 0.7 h for doses over 40 mg. In fact, results demonstrated lower values (around 7.2 h) for doses of 20 and 40 mg. This difference can be explained by the very low plasma concentrations obtained in the last phase of elimination of TA-3090 with many volunteers: they were below the limit of sensitivity of the method. This could account for the 2-level curve better than non-linearity that would give a constant increase.

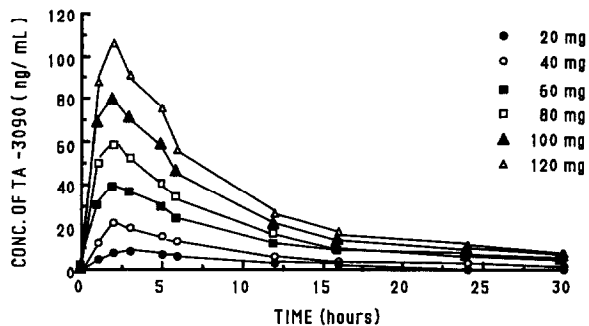
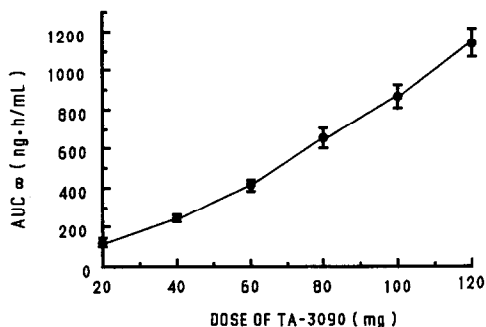


Figure 3
Mean plasma concentrations of TA-3090 after single oral doses of 20–120 mg in healthy volunteers.

Figure 4
Mean areas under the plasma concentration time curve of TA-3090 for oral doses of 20–120 mg in healthy volunteers.



From this study, it can be concluded that TA-3090 presents linear pharmacokinetics in man.

Conclusion

The proposed method of analysis is sensitive and reproducible and metabolites can be separated from TA-3090 and assayed. The method can be used to evaluate the pharmacokinetics of TA-3090 and future studies will be conducted to examine the correlation between pharmacodynamic effects and pharmacokinetic parameters.

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