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Validation of a chromatographic method to determine E-6006 and its metabolite E-6332 in rat and dog plasma by solid-phase extraction and capillary gas chromatography. Application in pharmacokinetics

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Abstract

E-6006, 5-{α-[2-(dimethylamino)ethoxy]-2-thienylmethyl}-1-methyl-1*H*-pyrazole is a new antidepresive compound and E-6332, 5-{α-[2-(methylamino)ethoxy]-2-thienylmethyl}-1-methyl-1*H*-pyrazole is its desmethylate metabolite. With the aim of quantifying E-6006 and E-6332, simultaneously in rat or dog plasma, a method of analysis based on solid-phase extraction coupled with capillary gas chromatographic system with N–P detection was developed and validated. E-6006, E-6332 and its internal standard (E-4018) were isolated from plasma using an off-line semiautomatic solid-phase extraction method. Gas chromatography separations were carried out by means of 12 m length, 0 2 mm (i.d.) and 0.33 μm (f.t.) ULTRA 1 type capillary column in splitless mode of injection at 190°C, with a TSD or specific nitrogen–phosphorus detector. No peaks interfering with the quantification of E-6332 and E-6006 were observed. The limit of quantification was 5 ng/ml with a precision and accuracy $<17\%$. The peak height ratios were proportional to E-6332 and E-6006 concentration over the range from 5 to 600 ng/ml ($r^2 > 0.998$). Mean recoveries of E-6332, E-6006 and internal standard from rat plasma were between 57.1 and 82.6. Intra-assay precision coefficients were <8.0 and $<11.8\%$, respectively, for E-6332 and E-6006, with an accuracy <12.6 and $<9.7\%$. Both inter-assay precision and accuracy were within acceptable limits ($<15\%$). In dog, the results were very similar to those obtained in rat. To show an example of the suitability of the method to determine E-6332 and E-6006, plasma profiles obtained after single oral and intravenous administration of 20 mg/kg to rats and 25 mg/kg to dogs are reported. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: E-6006; Solid-phase extraction-GC-NPD; Validation

1. Introduction

E-6006, 5-{α-[2-(dimethylamino)ethoxy]-2-thienylmethyl}-1-methyl-1*H*-pyrazole is an antidepre-

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sive compound and E-6332, 5-{α-[2-(methylamino)ethoxy]-2-thienylmethyl}-1-methyl-1*H*-pyrazole is its desmethylate metabolite. Both are products sintetized in our laboratory (Fig. 1).

E-6006 shows antidepressant activity in several animal models such as: Inhibition of Ptosis Induced by Reserpine in mouse [1] (when administered at 160, 80, 40, 20 and 10 mg/kg, p.o., showed a reverting activity of 83, 71, 66, 55 and 14%, respectively); Water Despair Test in rat [2], where the animal is forced to swim in a cylinder full of water from which it cannot escape, then after a short period of great activity, where the animal tries to escape from the cylinder, the rat remains motionless and does no attempt any further escaping movements, just those necessary to keep its head above water; antidepressants shorten the time of immobility as compared to the control group, being our product less active than others alike; and the reduction of Rat Pup Ultrasonic Vocalization [3], based on the fact that lactating rats emit ultrasonic vocalizations when they are isolated from their mother and litter, then when administered at 60 mg/kg by subcutaneous route a decrease in the number of ultrasonic vocalizations was observed, similar to the effect caused by diazepam at 0.5 mg/kg.

After studying the binding of E-6006 for many receptors, the compound did not show significant affinity for any of them.

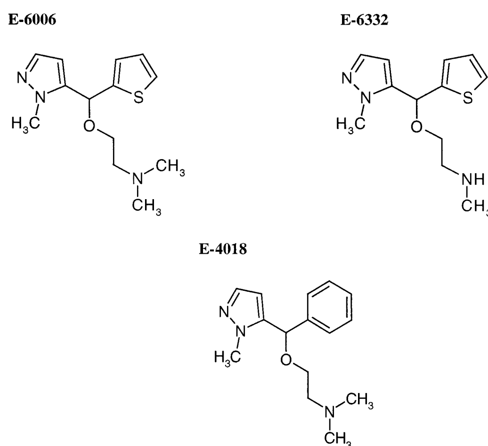

Fig. 1. Chemical structure of the compounds.

The aim of our work was to develop a sensitive and rapid method to quantify E-6006 and E-6332, simultaneously, in rat or dog plasma, using a method of analysis based on solid phase extraction followed by capillary gas chromatography with N–P detection.

2. Experimental

2.1. Chemicals and reagents

E-6006, 5-{α-[2-(dimethylamino)ethoxy]-2-thienylmethyl}-1-methyl-1*H*-pyrazole and its demethylate metabolite, E-6332, 5-{α-[2-(methylamino) ethoxy]-2-thienylmethyl}-1-methyl-1*H*-pyrazole were provided by Departament of Syntesis of Lab. Dr Esteve. The internal standard E-4018, cizolirtine citrate, 5-{α-[2-(dimethylamino)ethoxy] benzyl}-1-methyl-1H-pyrazole citrate was provided by Esteve Química, S.A. (Girona, Spain). Triethylamine and methanol were supplied by Scharlau (Barcelona, Spain). Demineralized water was purified in a MilliQ filtration system (Millipore Corporation, Bedford, Ma) to obtain water of HPLC grade. Drug-free rat plasma was obtained in our laboratory and that of dog was supplied by Centro de Investigación y Desarrollo Aplicado S.A. (Barcelona, Spain), both stored at −80°C until the assay.

2.2. Instrumentation

Chromatographic separations were performed using a Varian equipment (California, USA), consisting of a model Gas Chromatographer model 3400, a nitrogen–phosphoruos detector (TSD Bead Probe) and automatic injector model 8200. The software used to acquire the chromatograms was Access* Chrom supplied by Perkin Elmer (Cupertino, CA). The chromatograms were kept as data processing files.

The sample extraction system consisted of a Vac Elut SPS24 manufactured by Varian and an evaporating bath Turbo Vap LV, model ZW 7001 supplied by Zimark (Hopkinton, USA)

2.3. Chromatographic conditions

The cromatographic separation was performed on an Ultra 1 Crosslinked Methyl Siloxane, (12 m × 0.2 mm I.D. × 0.33 μm film thickness) purchased form Hewlett-Packard (Waldbronn, Germany). Gas (Carburos Metálicos, Barcelona, Spain) flow rates, as measured at the detector were: helium (carrier, 0.9–1 ml/min), air (160 ± 20 ml/min) and hydrogen (4.25 ± 0.2 ml/min). The helium source was protected by an oxy-clear and an omi-purifier (both from Supelco, Bellefonte, USA).

The injector and detector temperatures were set at 190 and 300°C, respectively, while in the oven the temperature gradient was as follows: an isothermal equilibration time of 3 min at 100°C, the first ramp ran from 100 to 170°C at a rate of 10°C/min; the second ramp then continued from 170 to 175°C at a rate of 0.5°C/min, finally, the third ramp was from 175 to 176°C at a rate of 20°C/min with a total run time of 20 min. The injection mode was splitless by means of an insert liner 'open insert' type, and the volume injected was 1 μl.

2.4. Preparation of stock solutions and working standard solutions

A stock solution of E-6006 and E-6332 (100 μg/ml of free-base) was prepared by dissolving 17.2 of E-6006 citrate and 13.6 mg of E-6332 oxalate in 100 ml of water. Drug concentrations in the working standard solutions chosen for the calibration curve were 0.1, 0.2, 0.8, 1.6, 2, 4, 8 and 12 μg/ml. These working solutions were made by further dilution of the stock solution with water.

A stock solution of internal standard (100 μg/ml) was prepared by dissolving 17.4 mg of E-4018 (cizolirtine citrate) in 100 ml of water, from which a working standard solution of 10 μg/ml was made. All the solutions were prepared daily.

2.5. Preparation of plasma standards and samples

The frozen drug-free rat or dog plasma was thawed at room temperature, vortexed and centrifuged at 2000 g for 10 min prior to use. Plasma standards and calibration standards for validation were prepared by adding 25 μl of each working solution to aliquots of 475 μl of rat or dog plasma. Then, 25 μl of internal standard (10 μg/ml) and 1 ml of a 0.08% triethylamine water solution were added and the vials vortexed vigorously about 1 min.

In the pharmacokinetic studies aliquots of 500 μl of plasma were pipetted into vials. Afterwards, the samples were treated as described above. Quality control samples were prepared by spiking drug-free rat or dog plasma with different working standard solutions of E-6006 and E-6332.

2.6. Solid-phase extraction

Solid-phase extraction of the samples was made on disposable C_2 cartridges of Bond Elut (3 cc volume/200 mg sorbent) supplied by Varian.

The procedure was as follows:

1. Before loading the above mentioned mixture into the cartridge, this was activated with 2 × 2.5 ml of methanol and 2 × 2.5 ml of a 0.08% triethylamine water solution.
2. The mixture was loaded into the cartridge.
3. Then, as washing phase, 2.5 ml of distilled water were passed through the cartridge to get rid off endogenous substances, proteins, and other polar components, thus increasing the selectivity of the method.
4. After the vacuum-drying phase (1 min) the products were eluted by flushing 5 ml of methanol through the column.
5. The 5 ml of eluate were evaporated to dryness under nitrogen flow and at 40 ± 2°C in the Turbo Vab evaporating bath.
6. Finally, the dry residue was reconstituted with 50 μl of methanol and vortex-mixed, and transferred to 200 μl glass vials for chromatography. An aliquot (1 μl) was injected into the gas chromatograph/NPD connected to a PE Nelson data-acquisition system.

2.7. Validation

The following parameters were determined for the validation of the analytical method developed for E-6006 and E-6332 in rat or dog plasma:

selectivity, linearity and range, precision, accuracy, limit of quantification, recovery and stability [4].

2.8. Pharmacokinetic application of the method

The validated method has been applied to pharmacokinetic studies in which the concentration of E-6006 and E-6332 were determined simultaneously in more than 100 plasma samples of rat, and in more than 200 plasma samples of dog. The former was carried out in our laboratory and consisted of an intravenous (under fasting conditions) and oral administration of E-6006 at a dose of 20 mg/kg; while the latter, whose administration and blood extraction were performed in Centro de Investigación y Desarrollo Aplicado S.A. (Barcelona, Spain), and the analysis in our laboratory, consisted also of an intravenous (under fasting conditions) and oral administration of E-6006 at a dose of 25 mg/kg.

3. Results and discussion

3.1. Selectivity

The selectivity of the method was determined by injecting drug-free plasma of eight different dogs (in rat eight samples of a same pool were used) [5]. The chromatograms thus obtained were free of interferences at the retention times of E-6006, E-6332 or internal standard. Furthermore, the pre-dose samples of the rat and dog pharmacokinetic studies did not show either any relevant interference (Fig. 2). The retention times in rat were 14.658 for E-6332, 14.909 for E-6006 and 15.165 for internal standard, being very similar in dog.

3.2. Linearity and range

The linear range for E-6006 and E-6332 in rat and dog plasma was validated using eight standards covering the range from 5 to 600 ng/ml.

E-6006 or E-6332-to-standard internal peak height ratios were plotted against the corresponding concentrations. Data were fitted to the equation $y = mx + b$, where y is the peak height ratio, x is the drug concentration ratios and m and b are the slope and y-intercept of the calibration curve, respectively (Table 4).

The calibration curves obtained during 8 days showed a linear relationship with a mean determination coefficient in rat of 0.9991 and 0.9992, and in dog of 0.9973 and 0.9985, for E-6332 and E-6006, respectively. Since the back-calculated values for these curves improved when they were forced through the origin, y-intercept value was always zero.

The linearity of the calibration curves was demonstrated by fitting the data comprising each curve to the equation $y = mx^N + b$ [6,7], and checking that the value of N was not different from one. The confidence interval (CI) associated to N was calculated according to the following equation:

$$\mathrm{CI} = N \pm t_{\alpha/2,\,\mathrm{df}} \times \mathrm{SE}$$

where N is the exponent of the equation; $t_{\alpha/2,\mathrm{df}}$ is the student's t distribution for the one-tailed probability level of 95% ($\alpha/2 = 0.025$) with $n-3$ degrees of freedom from error; SE is the standard error corresponding to the exponent and n is the number of points included in the calibration curve.

The confidence intervals obtained for each calibration curve included the unity suggesting that the method proposed to determine E-6006 and E-6332 in both rat and dog plasma is linear in the concentration range studied (5–600 ng/ml).

Back-calculated values for the calibration standards of the method in rat an dog plasma are presented in Table 5. The RSD (RSD = SD/mean × 100) ranged in rat between 1.6 and 9.2%for E-6332 and between 0.4 and 10.1% for E-6006, while in dog ranged between 1.5 and 8.8% for E-6332 and between 1.2 and 11.2% for E-6006.

3.3. Precision and accuracy

The precision of the assay for E-6006 and E-6332 was evaluated by determining the intraday and interday RSD of the measured peak height ratios of different concentrations. The intraday

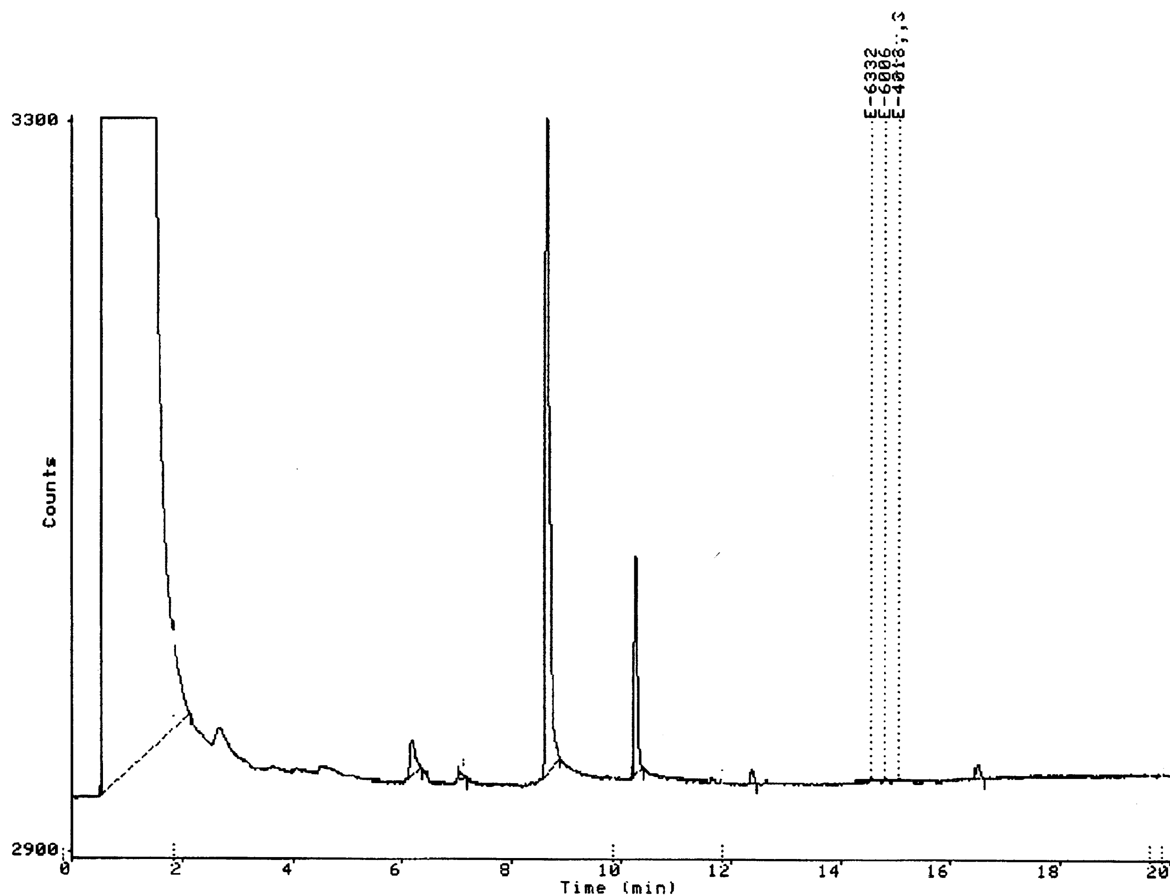

Fig. 2. Drug-free dog plasma.

precision of the method was determined by measuring eight plasma standards at four concentrations (5, 10, 100 and 600 ng/ml). These plasma standards were different from the calibration standards to avoid the influence of the calibration curve [8,9]. The results are presented in Table 1. The values obtained were in all cases lower than 11.8 and 10.5% in rat and dog, respectively. The interday precision was evaluated at the same four concentrations above mentioned during 8 days. The values obtained were very similar for both products and ranged from 3.0 to 16.9% in rat and from 2.6 to 11.3 in dog. As expected, the RSD increases as the concentration levels of E-6006 and E6332 decreases (Table 2). Both intraday and interday precision values for E-6006 as well as for E-6332 fell within the limits considered as acceptable (precision (RSD $<$ 15%) and accuracy (85–115%), but when coinciding with the limit of quantitation, precision (RSD $<$ 20%) and accuracy (80–120%)) [5].

The intraday and interday accuracy of the assay were calculated from the comparison of E-6006 or E-6332 concentrations determined in plasma standards with the corresponding nominal values. The accuracy was expressed as mean percentage of analyte recovered in the assay (Accuracy = (|calculated value-nominal value|/ nominal value) $\times$ 100). Table 1 shows the intraday accuracy ($n = 8$) evaluated at four concentrations (5, 10, 100 and 600 ng/ml). The values obtained in rat ranged between 1.4 and 12.6% for E-6332 and

between 1.7 and 9.7% for E-6006, whereas in dog ranged between 2.5 and 12.4% for E-6332 and between 2.8 and 5.3 for E-6006. Table 2 shows the interday accuracy ($n = 8$) evaluated at four concentrations (5, 10, 100 and 600 ng/ml). The values obtained in rat ranged between 2.6 and 12.7% for E-6332 and between 2.4 and 19.0% for E-6006, whereas in dog ranged between 2.4 and 9.9% for E-6332 and between 2.9 and 8.7 for E-6006. As well as in precision, all the values obtained for accuracy were within the limits considered as acceptable for bioanalysis [5].

3.4. Limit of quantitation (LOQ)

The limit of quantitation, defined in the presented study as the lowest plasma concentration in the calibration curve that can be measured routinely with acceptable precision (RSD < 20%) and accuracy (80–120%), was 5 ng/ml (Tables 1 and 2).

Table 1
Intraday-precision and accuracy of E-6332 and E-6006 assay in rat an dog plasma [a]

Animal species	n	Nominal concentration (ng/ml)	Precision				Accuracy	
			E-6332		E-6006		E-6332	E-6006
			Mean ± S.D.	RSD	Mean ± S.D.	RSD	Mean relative errors (%)	
Rat	8	5	111.8 ± 9.0	8.0	95.6 ± 11.2	11.8	12.6	9.7
	8	10	103.9 ± 7.2	6.9	90.3 ± 6.8	7.5	5.5	9.7
	8	100	101.2 ± 1.5	1.4	100.0 ± 2.1	2.1	1.4	1.7
	8	600	104.7 ± 4.3	4.1	102.9 ± 1.3	1.2	4.7	2.9
Dog	7	5	108.5 ± 10.2	9.4	95.0 ± 10.0	10.5	12.4	5.3
	8	10	103.8 ± 6.1	5.8	100.5 ± 5.0	5.0	5.8	4.3
	8	100	103.4 ± 4.3	4.2	99.0 ± 3.1	3.1	3.7	2.8
	8	600	102.5 ± 2.1	2.0	105.2 ± 1.4	1.4	2.5	5.2

[a] S.D., Standard deviation; RSD, Relative standard deviation (%); Relative errors, | calculated value − nominal value|/nominal value*100.

Table 2
Interday-precision and accuracy of E-6332 and E-6006 assay in rat an dog plasma [a]

Animal species	n	Nominal concentration (ng/ml)	Precision				Accuracy	
			E-6332		E-6006		E-6332	E-6006
			Mean ± S.D.	RSD	Mean ± S.D.	RSD	Mean relative errors (%)	
Rat	8	5	111.7 ± 14.4	12.9	108.9 ± 18.3	16.9	12.7	19.0
	8	10	104.3 ± 7.4	7.1	102.0 ± 13.9	13.7	6.6	11.9
	8	100	99.3 ± 3.5	3.5	100.6 ± 4.1	4.1	2.6	3.9
	8	600	99.7 ± 4.4	4.5	100.6 ± 3.0	3.0	3.3	2.4
Dog	8	5	106.6 ± 10.8	10.2	103.3 ± 10.0	9.7	9.3	8.7
	8	10	105.2 ± 11.9	11.3	108.0 ± 6.6	6.1	9.9	8.6
	8	100	101.1 ± 4.9	4.8	101.5 ± 5.4	5.3	3.8	4.3
	8	600	101.4 ± 2.6	2.6	100.7 ± 3.6	3.6	2.4	2.9

[a] S.D., Standard deviation; RSD, Relative standard deviation (%).

Table 3
Recovery of E-6332, E-6006 and its internal standard from rat an dog plasma [a]

Animal species	n	Nominal concentration (ng/ml)	Recovery (%)					
			E-6332		E-6006		E-4018 (I.S)	
			Mean ± S.D.	RSD	Mean ± S.D.	RSD	Mean ± S.D.	RSD
Rat	6	10	67.3 ± 5.8	8.6	82.6 ± 7.8	9.4	–	–
	8	100	57.1 ± 1.6	2.8	59.0 ± 1.4	2.3	–	–
	8	600	67.0 ± 2.3	3.4	74.1 ± 2.5	3.4	–	–
	8	500	–	–	–	–	82.6 ± 3.6	4.3
Dog	8	10	71.2 ± 8.4	11.7	69.8 ± 13.9	20.0	–	–
	8	100	77.1 ± 2.9	3.8	76.1 ± 5.0	6.6	–	–
	8	600	71.0 ± 3.8	5.4	72.0 ± 4.3	6.0	–	–
	8	500	–	–	–	–	83.6 ± 3.9	4.7

[a] S.D., Standard deviation; RSD, Relative standard deviation (%).

Table 4
Linearity of the calibration curves obtained in E-6332 and E-6006 assay in rat and dog plasma $y = mx + b$ [a]

	n	Concentration range (ng/ml)	r^2		m	
			E-6332 mean ± S.D.	E-6006 mean ± S.D.	E-6332 mean ± S.D.	E-6006 mean ± S.D.
Rat	8	5–600	0.9991 ± 0.0007	0.9992 ± 0.0006	0.9884 ± 0.0537	1.0046 ± 0.0592
Dog	8	5–600	0.9973 ± 0.0024	0.9985 ± 0.0011	0.9955 ± 0.1995	0.9839 ± 0.0157

[a] S.D., Standard deviation; Curves forced through the origin, $b = 0$.

3.5. *Recovery*

The percentage of E-6006 and E-6332 recovered from plasma using the proposed procedure, was calculated by comparison of the drug peak height ratios in the extracted plasma ($n = 8$) with the mean peak height ratios obtained form direct injection of the corresponding unextracted standard solutions. The recovery was measured at three different concentration (10, 100 and 600 ng/ml) over the calibration range used. As for the internal standard, recovery was only calculated at the working concentration (500 ng/ml).

Table 3 shows the recovery, expressed as percentage, obtained for E-6006, E-6332 and internal standard. The values found for both products are regardless of the drug concentration, and the ranges obtained in dog are narrower than those in rat, being those in rat between 57.1 and 67.3% for E-6332, and between 59.0 and 82.6% for E-6006; while in dog are between 71.0 and 77.1% for E-6332, and between 69.8 and 76.1% for E-6006. No clear relationship between concentration and recovery was found. For the internal standard ($n = 8$), a recovery of 83% was obtained in both species.

The explanation for the fact that the recovery for both products are lower than that for the internal standard, is that actually this assay was designed to determine E-4018, a very similar compound, which now is our current internal standard, and all changes we tried to improve the recovery were unsuccessful, so the procedure remained unmodified.

3.6. Stability

The stability study is currently in process (storage conditions in each plasma at − 30 and − 80°C, and freeze/thaw cycles). However, we have some date of stability of both products and the internal standard, stored at 4°C for 1 week as methanolic and water solutions. The results showed that this storage was possible since these were in methanol 97.2 ± 5.3, 101.0 ± 5.4 and 98.4 ± 4.9%, and in water 99.5 ± 2.6, 101.5 ± 2.1 and 103.3 ± 3.2%, respectively for E-6332, E-6006 and internal standard. All the values are expressed as percentage found ± SD.

3.7. Application of the method

This procedure has been applied to the analysis of samples from two pharmacokinetic studies, one in rat, and another in dog.

In these studies, rat or dog plasma samples together with calibration standards and quality control samples were assayed for E-6006 and E-6332 content (Figs. 3 and 4).

Drug concentrations determined in the quality control samples were in good agreement with the nominal concentrations, since the percentage of quality control acceptation in both species was superior to 90%. Furthermore, the back-calcu-

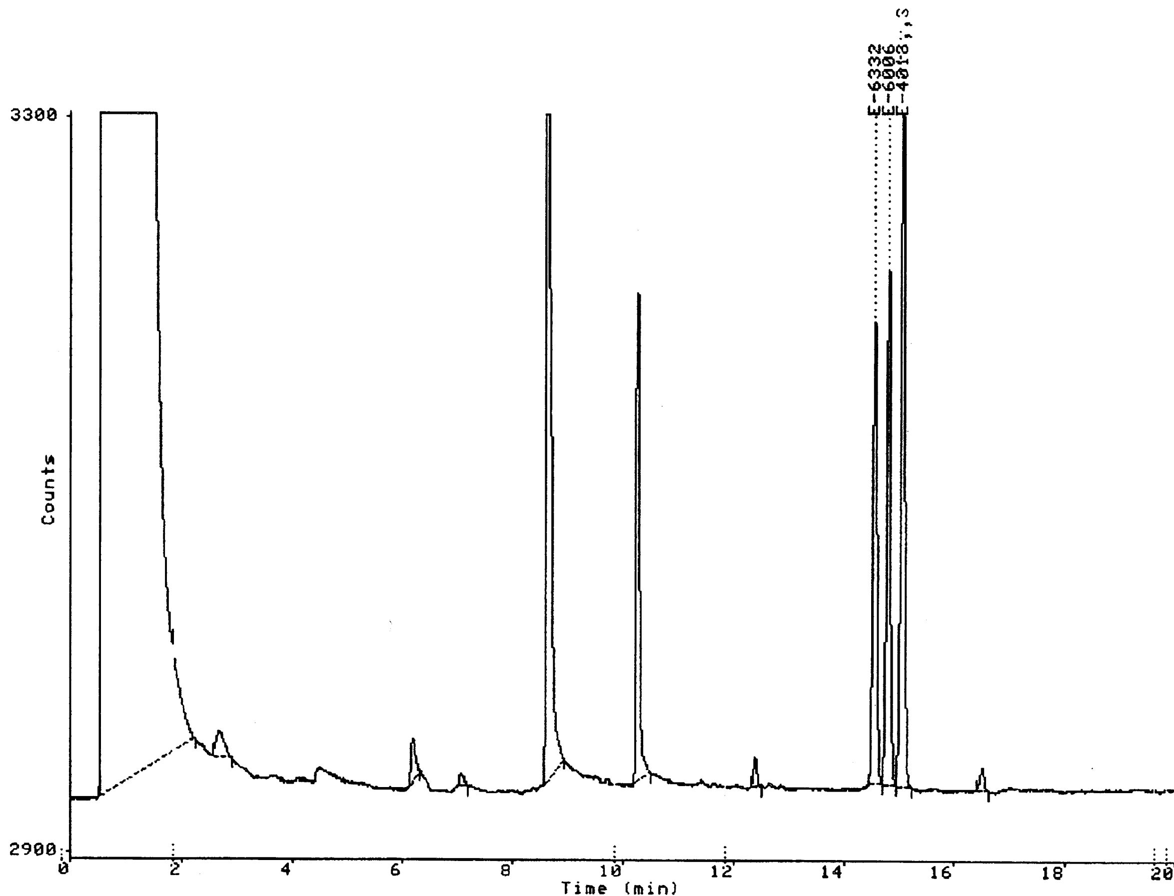

Fig. 3. Drug-free dog plasma spiked with 400 ng/ml of E-6332 and E-6006, and E-4018 (500 ng/ml).

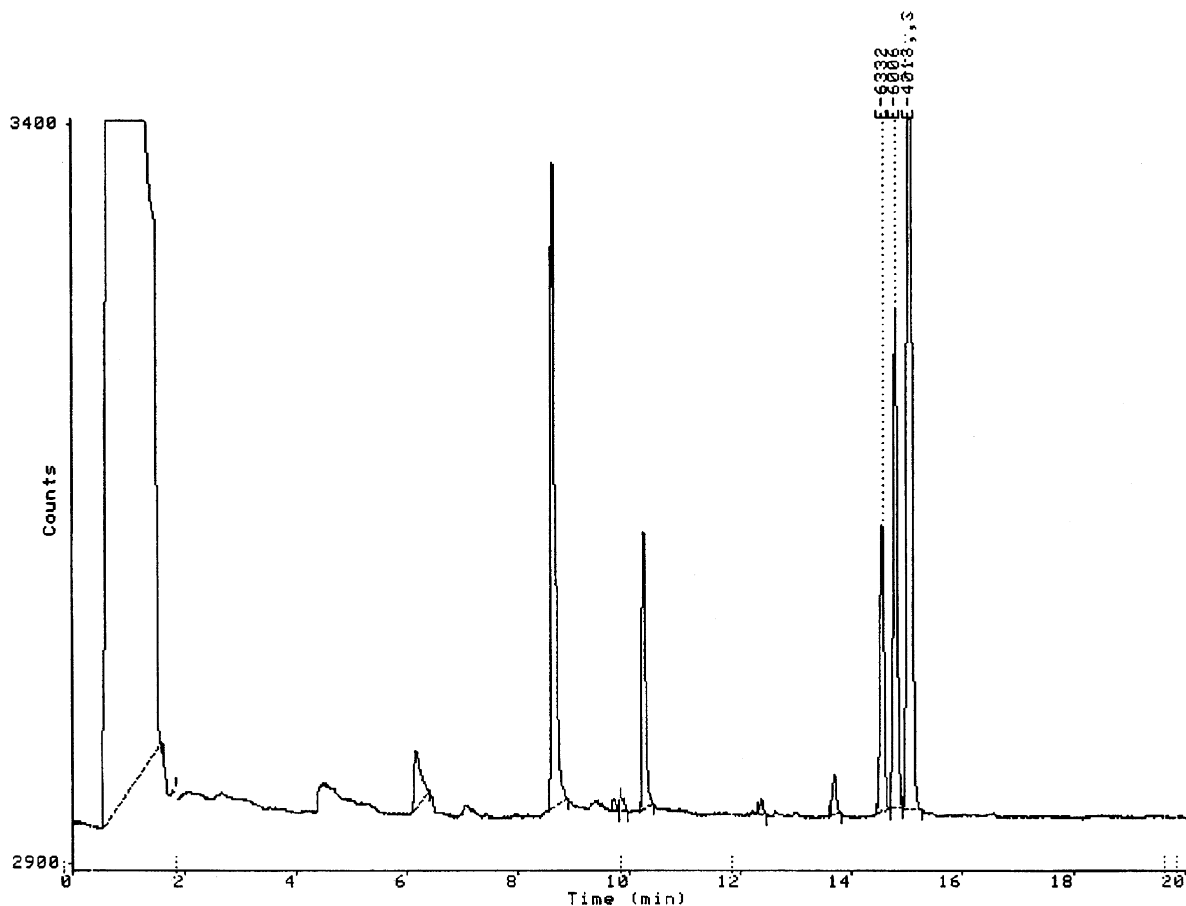

Fig. 4. Plasma sample from one dog collected 3 h after oral administration of 25 mg/kg E-6006.

and in dog 100.9 ± 6.8 and $99.5 \pm 5.5\%$, for E-6332 and E-6006, respectively (Table 5).

4. Conclusions

The method to determine simultaneously E-6006 and E-6332 (its desmethylate metabolite) in rat or dog plasma described in this study was found to be suitable to determine concentrations in the range from 5 to 600 ng/ml precisely and accurately. The recovery of both products was not high but acceptable, and the method has been successfully used in pharmacokinetic studies both in rat and dog. The percentage of quality control acceptation in both species was superior to 90%, and the back-calculated values for the calibration standards of the method were found to be between 95 and 101% for both products, what shows how successful this method has become to determine E-6006 and E-6332.

Table 5
Back-calculated concentrations (Mean ± SD) of calibration samples ($n = 8$) [a]

Concentration added (ng/ml)	Concentration found (ng/ml)							
	Rat				Dog			
	E-6332 mean	RSD	E-6006 mean	RSD	E-6332 mean	RSD	E-6006 S.D.	RSD
5	5.4 ± 0.5	9.2	4.6 ± 0.5	10.1	5.5 ± 0.3	5.5	5.0 ± 0.6	11.2
10	10.2 ± 0.6	5.7	9.5 ± 0.1	1.3	10.5 ± 0.9	8.8	10.2 ± 0.7	6.4
40	36.5 ± 2.0	5.5	36.7 ± 0.4	1.0	39.8 ± 1.5	3.8	40.2 ± 1.8	4.5
80	76.6 ± 4.8	6.3	76.9 ± 0.8	1.0	78.3 ± 4.4	5.6	79.5 ± 3.3	4.1
100	96.0 ± 5.8	6.0	92.7 ± 2.3	2.5	95.8 ± 5.9	6.1	95.8 ± 5.1	5.3
200	187.5 ± 9.1	4.8	197.6 ± 1.8	0.9	198.5 ± 9.4	4.7	197.0 ± 3.9	2.0
400	391.3 ± 15.7	4.0	384.5 ± 3.7	1.0	391.4 ± 11.3	2.9	426.5 ± 11.7	2.7
600	610.0 ± 9.7	1.6	611.8 ± 2.2	0.4	606.4 ± 9.3	1.5	603.5 ± 7.4	1.2

[a] S.D., Standard deviation; RSD, Relative standard deviation (%).

Table 5
Back-calculated concentrations (Mean ± SD) of calibration samples ($n = 8$) [a]

Concentration added (ng/ml)	Concentration found (ng/ml)							
	Rat				Dog			
	E-6332 mean	RSD	E-6006 mean	RSD	E-6332 mean	RSD	E-6006 S.D.	RSD
5	5.4 ± 0.5	9.2	4.6 ± 0.5	10.1	5.5 ± 0.3	5.5	5.0 ± 0.6	11.2
10	10.2 ± 0.6	5.7	9.5 ± 0.1	1.3	10.5 ± 0.9	8.8	10.2 ± 0.7	6.4
40	36.5 ± 2.0	5.5	36.7 ± 0.4	1.0	39.8 ± 1.5	3.8	40.2 ± 1.8	4.5
80	76.6 ± 4.8	6.3	76.9 ± 0.8	1.0	78.3 ± 4.4	5.6	79.5 ± 3.3	4.1
100	96.0 ± 5.8	6.0	92.7 ± 2.3	2.5	95.8 ± 5.9	6.1	95.8 ± 5.1	5.3
200	187.5 ± 9.1	4.8	197.6 ± 1.8	0.9	198.5 ± 9.4	4.7	197.0 ± 3.9	2.0
400	391.3 ± 15.7	4.0	384.5 ± 3.7	1.0	391.4 ± 11.3	2.9	426.5 ± 11.7	2.7
600	610.0 ± 9.7	1.6	611.8 ± 2.2	0.4	606.4 ± 9.3	1.5	603.5 ± 7.4	1.2

[a] S.D., Standard deviation; RSD, Relative standard deviation (%).

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