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Sensitive microanalysis of imipramine and desipramine in single rat thyroids by gas chromatography–mass spectrometry

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Abstract

A new sensitive method for the quantitative determination of imipramine and desipramine in single rat thyroids using gas chromatography–mass spectrometry with selected ion monitoring, after enzymatic hydrolysis and liquid–liquid extraction has been developed. The technique was deemed suitable for microanalysis of single rat thyroids and for other solid tissues, using smaller sample sizes than usually required for traditional determination methods. The quantification was linear from 10 to 200 nmol/l (i.e., from 0.25 to 5 μg/g) for imipramine and from 100 nmol/l to 2000 nmol/l (i.e., from 2.4 to 47 μg/g) for desipramine, and the limits of detection (less than 25 ng/g tissue for both compounds) were better than those previously reported. Recoveries, repeatability and reproducibility of this technique were satisfactory. It has been successfully applied in a preliminary study of the concentration–time profiles of imipramine and desipramine in the thyroid of rats treated with either of these drugs.

Keywords: Imipramine; Desipramine

1. Introduction

Imipramine and its demethylated major metabolite desipramine are both used as antidepressants in clinical practice of psychiatry. In recent years, different studies have reported that imipramine and desipramine may reduce the serum levels of thyroid in humans [1,2] and in rats [3–5]. In addition to the mechanisms usually proposed for the interaction between thyroid hormone levels concerning brain and CNS [5,6], a complementary mechanism could be an antithyroid mechanism based on complexation of molecular iodine in the thyroid [7,8], provided there is an accumulation of the drug in the gland. As a matter of fact, imipramine and desipramine are characterized, in vitro, by a high constant of iodine complexation [9]. Therefore, in order to validate this mechanism it had to be ascertained that these drugs penetrate and concentrate in the thyroid. Drug analysis in thyroid has rarely been reported and usually is concerned with antithyroid drugs (like methimazole, propylthiouracil). Most of the time, a single administration of a radio-labeled compound was used [10–13]. To date, several methods have been reported for the measurement of imipramine and its demethylated metabolite in biological fluids [14–19], and in sever-

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al tissues (but not in thyroid), by spectrophotometry [20], thin layer chromatography [21], radioisotope techniques [22,23], or HPLC [24–26]. Unhappily, all these methods used a relatively large mass of tissue, several times greater than a rat thyroid and were characterized by a very low sensitivity, unsuitable for rat thyroid analysis (except maybe for the method of Besret et al. [26], the detection limits of which were 25 ng/g and 20 ng/g for imipramine and desipramine, respectively). So, in order to determine the concentration–time elimination profile of imipramine and desipramine in thyroid this work intended to design and validate a sensitive method for the quantitative analysis of these drugs in single rat thyroids after repeated administrations.

2. Experimental

2.1. Chemicals and reagents

Imipramine hydrochloride and desipramine hydrochloride were provided by Sigma (St. Quentin Fallavier, France). Sodium hydroxide, isoamyl alcohol Normapur, chlorhydric acid Normapur, Na_2CO_3, $NaHCO_3$ were obtained from Prolabo (Paris, France). Heptane was purchased from Fisons (Paris, France) and toluene from Merck (Darmstadt, Germany). Trizma, bis[tris(hydroxymethyl)aminomethane] 99.9% (Tris base) and protease subtilisin Calsberg Type VIII bacterial from *Bacilius licheniformis* (subtilisin) were obtained from Sigma. Cyproheptadine (internal standard) was a gift of Merck Sharp (Paris, France). Methanolic solutions of imipramine, desipramine (1 mol/l), and of the internal standard (300 mg/l) were prepared and stored in colored glass vials, at 4°C; they were stable for at least one month. Heptane–isoamyl alcohol (98.5:1.5, v/v) and toluene–isoamyl alcohol (8.5:1.5, v/v) were also prepared in colored glass vials. Carbonate buffer (pH 9.7) was prepared by mixing 357 ml of 1 *M* Na_2CO_3 and 643 ml of 1 *M* $NaHCO_3$. Tris buffer (pH 7.5±0.3) was prepared every week from 0.2 *M* Tris base, 0.2 *M* HCl and deionized water (25:20.7:54.3, v/v/v). A 500 mg/l subtilisin stock solution in this Tris buffer was kept for one week, a 20 mg/l working solution in Tris buffer was prepared every day by dilution.

2.2. Chromatography

The assay was performed on a Hewlett-Packard 5890 Series II gas chromatograph coupled to a Hewlett-Packard 5972 mass-selective detector. A Supelco PTE5 (30 m×0.25 mm I.D., 0.25 μm film thickness) capillary column was used with helium as carrier gas, delivered at a column head pressure of 1 kg/m^2. The injector temperature was 270°C. The initial oven temperature was 110°C, increased to 220°C at a rate of 40°C/min, and to 230°C at 1°C/min. The total run time was 12.75 min. The gas chromatograph interface temperature was held at 280°C. The detector was operated in the selected-ion monitoring (SIM) mode, after 70 eV electron-impact ionization. Selected m/z ratios were 234 for quantification, 195 and 280 for confirmation of imipramine, 195, 234 and 266, respectively for desipramine, they represent the molecular ion and two prominent high mass fragments for each compound. The mass spectra and the structures of both compounds are presented Fig. 1. The entire process, including data acquisition and analysis, was controlled by HPCHEM software on a Hewlett-Packard Vectra 486 microcomputer.

2.3. Extraction of imipramine and desipramine from rat thyroid

Each thyroid was ground in a 5 ml Prolabo mortar, with 0.5 ml Tris buffer (pH 7.5±0.3) and 1 ml of subtilisin (20 mg/l). The samples were sonicated in 15 ml round-bottom glass tubes, at ambient temperature for 10 min. The enzymatic hydrolysis was performed during 1 h 45 min at 55°C in a water-bath. Then, 100 μl of the 3 mg/l internal standard solution, 1 ml of 0.25 *M* NaOH and 7.2 ml of heptane–isoamyl alcohol was added to 1 ml of supernatant in another 15 ml glass tube. The tubes were shaken for 15 min on a Laboral oscillating agitor (Prolabo), centrifuged at 2000 *g* for 15 min. Then the organic phase (5 ml) was transferred in a third 15 ml glass tube containing 1.2 ml of 0.1 *M* HCl. After 15 min shaking and 5 min centrifugation, the aqueous phase was made alkaline with 1 ml of carbonate buffer (pH 9.7) and the drugs extracted with 50 μl of toluene–isoamyl alcohol. After another shaking and centrifugation, the aqueous phase was

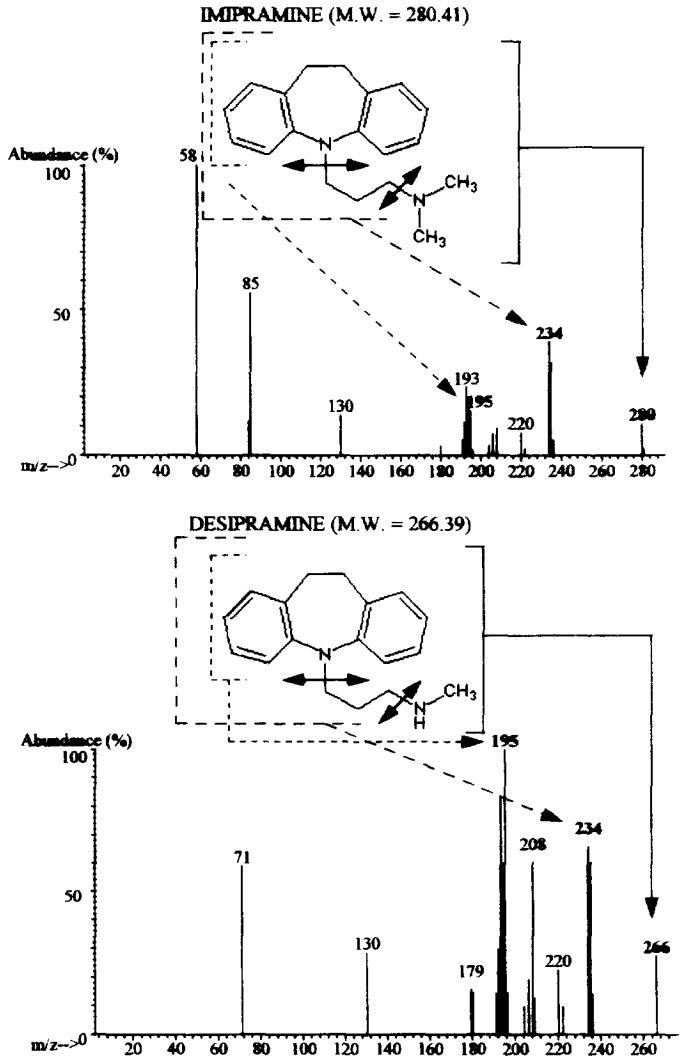

Fig. 1. Mass spectra and structures of imipramine and of desipramine.

discarded. 3 μl of the organic phase were injected in the chromatograph.

2.4. Standard curves and validation procedures

Due to the small amount of tissue obtained from one rat thyroid (and therefore to the great number of rats that would be necessary to validate the method using rat thyroids), the method validation was realized by spiking (by injection in the matrix) 17±2 mg sheep thyroid pieces (approximately the mass of a rat thyroid) with standards. Calibration graphs were constructed to achieve supernatant imipramine concentrations of 10, 30, 50, 75, 100, 150, 200 nmol/l (approximately equivalent to 0.88, 2.65, 4.41, 6.62, 8.82, 13.23, 17.65 nmol/g in thyroid, respectively) and supernatant desipramine concentrations of 100, 300, 500, 750, 1000, 1500, 2000 nmol/l (approximately equivalent to 8.8, 26.5, 44.1, 66.2, 88.2, 132.3, 176.5 nmol/g, respectively). The spiked calibration samples were submitted to the extraction procedure described in Section 2.3 and standard curves were generated by plotting drug to internal standard peak-area ratios against theoretical con-

centrations. Blank samples were also prepared and analyzed, in order to verify that no unknown peak resulting from the extraction procedure interfered with imipramine or desipramine. The detection limit of the method was defined as the lower concentration giving a signal-to-noise ratio greater than 3:1. Absolute recoveries were studied by spiking with 40 and 100 ng of drug (contained in 1 μl deionized water), (i) six pieces of sheep thyroid for each drug; (ii) six rat thyroids for desipramine; all these preparations were analyzed on the same day. The analytical procedure was as described in Section 2.2 Section 2.3, except that the internal standard was added just before the chromatographic injection. Analyte–internal standard areas ratios were compared to those obtained with non-extracted methanolic mixtures of the same theoretical concentration. The intra-day variability of the method was determined by analyzing six blank sheep thyroid pieces spiked with a same low or high concentration of each drug (imipramine: 20 and 100 nmol/l, i.e., about 1.76 and 8.82 nmol/g; desipramine: 250 and 1000 nmol/l, i.e., about 22.05 and 88.23 nmol/g). The precision was evaluated by the coefficient of variation of the areas ratio, for each level of each compound. The inter-day precision and accuracy of the method were evaluated by determining a different set of seven calibrating standards of each drug of interest, on four different days over a period of fourteen days. The linearity was studied from these same sample series.

2.5. Animals

The method described in Section 2.3 was used to determine imipramine and desipramine concentrations in the thyroid of rats treated by these drugs, in order to obtain a preliminary study of the concentration–time elimination profile. Male Wistars rats weighing 200±10 g were employed throughout. Two groups of nine rats each were administered imipramine hydrochloride (32 mg/kg per day) and desipramine hydrochloride (32 mg/kg per day), respectively, once daily (between 9 and 10 a.m.) for 4 weeks, by gavage. In each group, a single rat was killed at each of nine time intervals after the last administration of the drug: 1, 2, 3, 5, 7, 9, 11, 12 and 24 h. Its thyroid was removed, weighed and stored at −20°C until assay.

3. Results

Typical chromatograms obtained from a blank piece of sheep thyroid, a thyroid piece spiked with imipramine and desipramine and from thyroids of rats treated for four weeks, with 32 mg/kg per day of either HCl-imipramine or HCl-desipramine, are shown in Fig. 2. The retention times were 8.5 min for imipramine, 8.8 min for desipramine and 11 min for cyproheptadine (internal standard). The detection limit was lower than 1 nmol/l of supernatant for both drugs, or less than 0.09 nmol/g thyroid (i.e., less than 25 ng/g thyroid). The mean recoveries of imipramine and desipramine from pieces of sheep thyroid were 43.5±0.8% and 42.5±0.7%, respectively (mean±S.E.M.). From rat thyroid, the mean recovery of desipramine was 42.0±1.3%. Over the studied ranges, intra-day coefficients of variation were less than 10%. Inter-day coefficients of variation and relative difference were less than 10% with imipramine, and less than 20% with desipramine (except for 100 nmol/l where the coefficient of variation was slightly above 20%) (Tables 1 and 2). Based on inter-day study, the quantification limit would be less than 10 nmol/l for imipramine (less than 0.88 nmol/g thyroid) and approximately 100 nmol/l for desipramine (approximately 8.8 nmol/g thyroid). An analysis of variance of the results obtained from the four different calibration curves showed that the regression was significant ($p<1\%$) and the deviation from linearity non significant (for a 5% risk factor) for both drugs. The coefficients of correlation were 0.998 and 0.993, for imipramine and desipramine, respectively. Typically, the linear regression coefficients obtained by the least-squares were for imipramine: mean-slope 0.0052±0,0001, intercept 0.0154±0.0142; for desipramine: mean-slope 0.0033±0.0001, intercept −0.1295±0.0698.

Fig. 3 illustrates the results obtained in the preliminary study of concentration–time elimination profile observed in the 24 h which followed the last drug administration. In rats treated by imipramine, imipramine concentrations in thyroid varied between 1.5 and approximately 7 nmol/g, those of desipramine between 10 and 27.5 nmol/g. Desipramine treatment have led to more thyroid accumulation of drug, the observed concentrations being between 25 and 48.6 nmol/g.

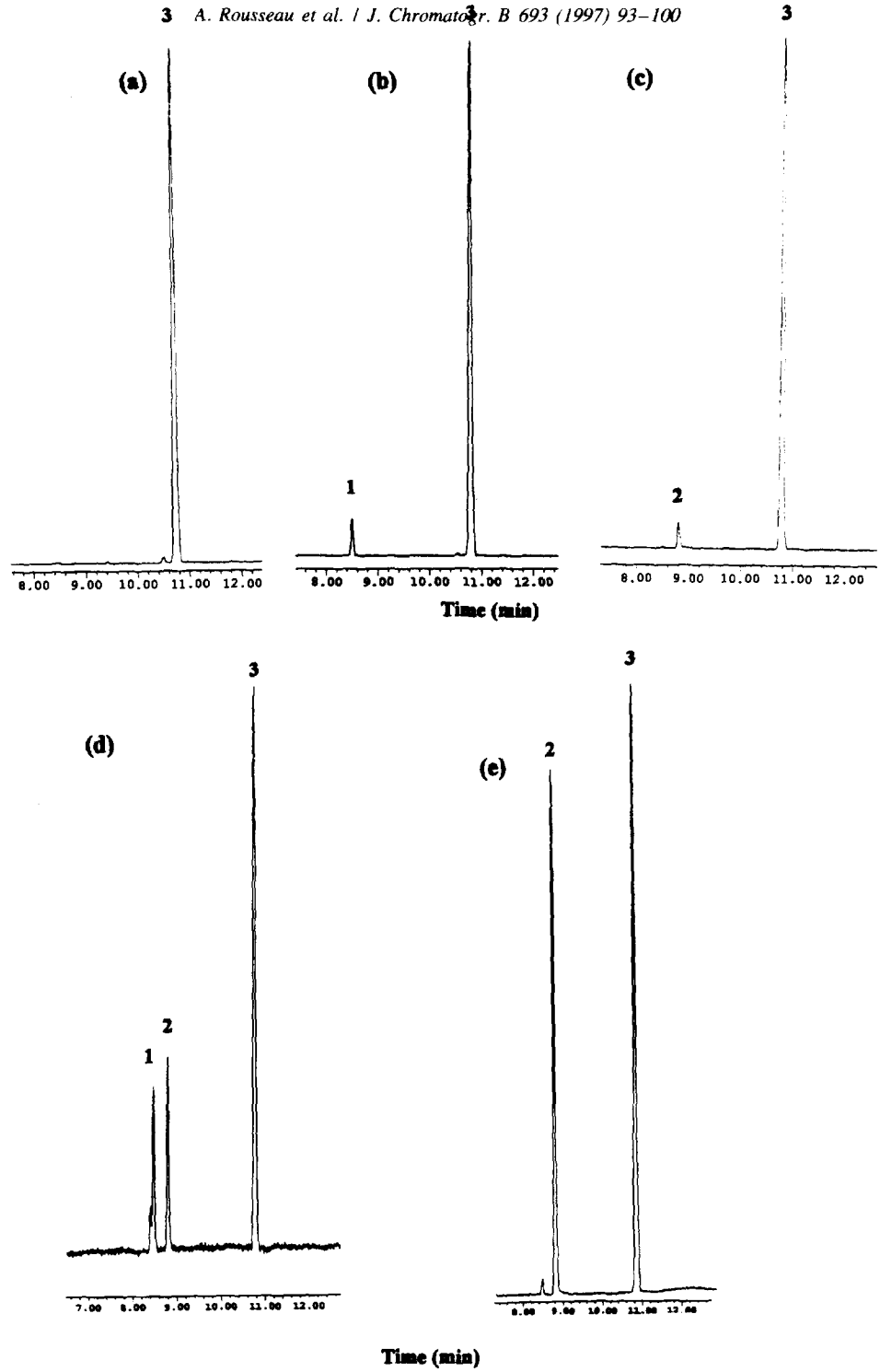

Fig. 2. Typical chromatograms: extract of a blank piece of sheep thyroid (a); of a piece of sheep thyroid spiked with imipramine (b) or desipramine (c) at detection limit 1 nmol/l of supernatant; extract of a single rat thyroid 1 h after the last administration of imipramine (d) or desipramine (e), in rats treated for four weeks by 32 mg/kg per day of either HCl-imipramine or HCl-desipramine. Peaks: 1=imipramine (t_R=8.5 min); 2=desipramine (t_R=8.8 min); 3=cycloheptadine (t_R=10.8 min, internal standard).

Table 1
Intra-day reproducibility of imipramine and desipramine analysis in thyroid

Theoretical concentration – in supernatant nmol/l – (in the tissue nmol/g)	Mean found concentration (nmol/l in supernatant)	Relative difference (%)	C.V. (%)
Imipramine			
20 (≈1.75)	20.8	3.9	9.0
100 (≈8.8)	97.2	2.8	5.6
Desipramine			
250 (≈22.1)	233.3	6.7	9.6
1000 (≈88.2)	1052	5.2	7.9

4. Discussion

The present microanalysis method, using gas chromatography–mass spectrometry, was deemed suitable to quantitatively determine imipramine and desipramine in one rat thyroid; its applicability was demonstrated in the reported preliminary study of the concentration–time elimination profile of these drugs in the thyroid of rats, treated for four weeks, with 32 mg/kg per day of either HCl-imipramine or HCl-desipramine. The limits of detection of the technique were excellent, much better than usually reported [20–22,25], except one HPLC method which gave similar detection limits [26]. The real limits of quantification were probably even better than the values determined on base of inter-day study, since linear calibration graphs were constructed daily. The mean recoveries were low, but they were reproducible. The lost in recovery was probably due to the first extraction step, from the solid matrix. Several attempts were made to improve it, by modifying grinding and enzymatic hydrolysis conditions, but were unsuccessful. Usually, investigators did not add drugs into the solid matrix, but rather into homogenates or aliquots [22,24] where the extraction was probably easier, obtaining better apparent recoveries. Now, repeatability, reproducibility and accuracy of the present method were satisfactory.

The concentrations obtained in the preliminary study were in the validity range of the technique. These concentrations, determined from single thyroids of treated rats, were compatible with the values reported by other investigators in other solid tissues: (i) DMI concentrations of 11.44±1.08 μg/g (42.86±4.04 nmol/g) in rat brain were reported after chronic administration of DMI (15 mg/kg every 12 h for 4 days) and for a sacrifice at twelfth hour after the last dose [25]; as this drug has been shown to accumulate to a high degree in the brain, this consistency of concentrations for a nearly equal dose suggests that DMI accumulates at almost the same extent as in rat brain; (ii) in rats treated by imi-

Table 2
Inter-day reproducibility and accuracy of imipramine and desipramine analysis in thyroid

Imipramine				Desipramine			
Theoretical concentration (nmol/l in supernatant)	Mean found concentration (nmol/l in supernatant)	C.V. (%)	Relative difference (%)	Theoretical concentration (nmol/l in supernatant)	Mean found concentration (nmol/l in supernatant)	C.V. (%)	Relative difference (%)
10	0.7	10.1	7.4	100	104	20.8	4.2
30	28.6	16.9	4.5	300	281	15.1	6.3
50	53.8	12.8	7.7	500	508	11.4	1.6
75	73.9	8.5	1.4	750	655	12.3	12.6
100	96.9	10.5	3.1	1000	919	13.2	8.1
150	152.0	2.5	1.3	1500	1400	10.6	6.7
200	197.0	3.2	1.5	2000	2073	2.1	3.6

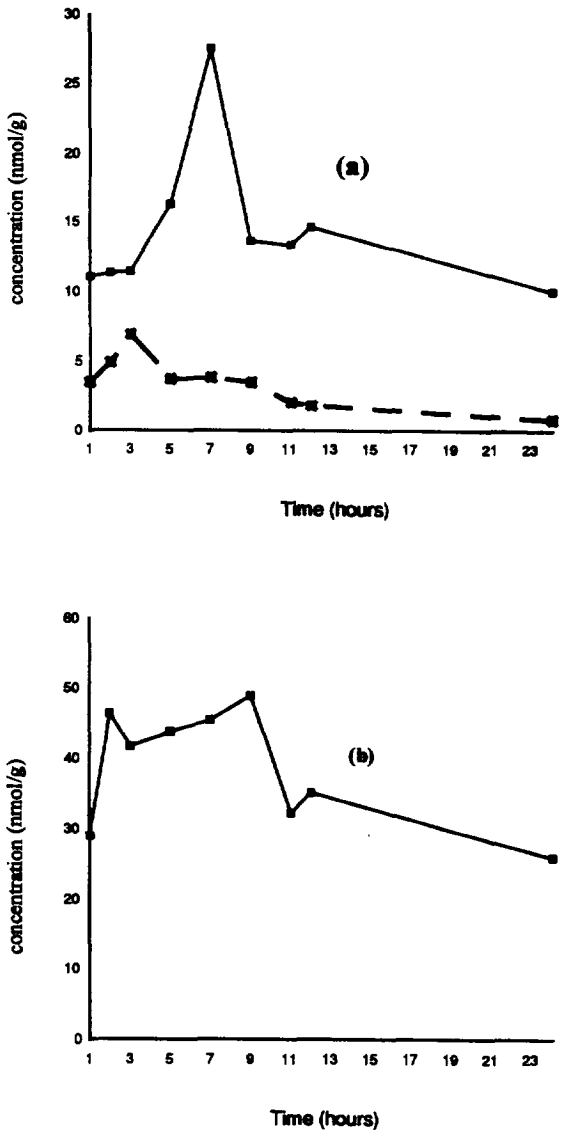

Fig. 3. Intra-thyroid concentration of imipramine (— — —) and desipramine (———) after administration to rats for 4 weeks, of 32 mg/kg/day of either HCl-imipramine (a) or HCl-desipramine (b).

pramine, reported concentrations of imipramine in brain [27] and in different tissues [21] were compatible with the concentrations in the thyroid of rats treated by imipramine determined in the present study; moreover, desipramine concentrations in brain were also higher than those of the parent drug [27].

In conclusion, a highly sensitive GC–MS method for quantitative determination of imipramine and desipramine from solid tissues was developed. It appeared suitable to characterize the pharmacokinetic disposition of imipramine and desipramine in single rat thyroid or in other situations where only small amounts of tissues are available. This method, using liquid–liquid extraction is also suitable for biological fluids [28].

More complete pharmacokinetic investigations, in rat thyroid and serum are in progress.

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