

# Spectrophotometric Determination of Trimipramine in Urine after Its Preconcentration into a Microdroplet Using Homogeneous Liquid-Liquid Extraction

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## Abstract

A simple, sensitive and reliable extractive-spectrophotometric method for the determination of trimipramine (TPM) in urine samples was developed. In a strong homogeneous acidic medium, trimipramine produced a colored compound which could be extracted into a sedimented microdroplet, in the presence of perfluorooctanoate ion (PFOA<sup>-</sup>) as a phase separator agent. The concentration of the extracted colored compound in the microdroplet was determined by measuring its absorbance at 390 nm after dilution with methanol. Under the optimal experimental conditions (Concentration of HNO<sub>3</sub> = 1.1 mol L<sup>-1</sup>, Concentration of PFOA<sup>-</sup> =  $7.7 \times 10^{-3}$  mol L<sup>-1</sup>, Concentration of THF = 19%), sample solutions containing 0.01 – 7 µg mL<sup>-1</sup> of TPM could be analyzed. The maximum concentration factor was 361 (13 mL sample solution produced a 36 µL sedimented liquid phase). The limit of detection of the proposed method was 1.2 ng mL<sup>-1</sup>. The reproducibility of the proposed method, for the determination of 1.92 µg mL<sup>-1</sup> of TPM, on 10 replicated measurements was at most 2.9%. The proposed method was successfully extended to the extraction and determination of trimipramine in pharmaceutical preparations and urine samples. The results showed good agreement to the certified analytical methods.

**Keywords:** Trimipramine, homogeneous liquid-liquid extraction, spectrophotometric determination, urine

## 1. Introduction

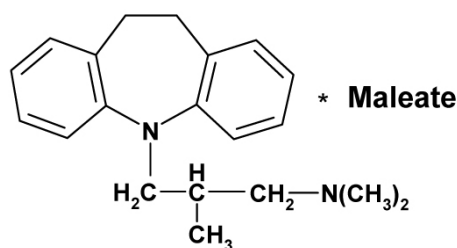
Homogeneous liquid-liquid extraction (HLLE) utilizes the phase separation phenomenon from a homogeneous solution and the target solutes are extracted into a microdroplet sedimented phase. The initial condition is a homogeneous solution and there is no interface between the water phase and the water-miscible organic solvent phase. It means that the surface area of the interface is infinitely large initially. Therefore, no vigorous mechanical shaking is necessary. The procedure is simple and requires only the addition of the reagent.<sup>1</sup> Similar phase separation phenomena such as the salting effect,<sup>2</sup> cloud point extraction,<sup>3</sup> and aqueous liquid-liquid extraction<sup>4</sup> are known as types of HLLE. Among them, the HLLE with perfluorosurfactants has significant advantages such as the possibility of achieving high concentration factors, short run time, and optional control of the volume of the sedimented phase at

microliter levels. These features could not be done using the salting effect, cloud point extraction, and aqueous two-phase extraction procedures. Therefore, the HLLE with perfluorosurfactants has been recently applied to the preconcentration of some organic and inorganic analytes.<sup>5–7</sup>

Trimipramine maleate (TPM), 5-(3-dimethylamino-2-methylpropyl)-10,11-dihydro-5H-dibenz [b,f] azepine acid maleate, is a tricyclic antidepressant agent with an anxiety-reducing and sedative activity that substantiates its efficacy in the treatment of primary insomnia. Due to its pharmacological profile, trimipramine might also be active as an antipsychotic. The therapeutic and pharmacological relevance of trimipramine, in addition to its inherent toxicity has prompted the development of several methods for its determination both in pharmaceutical preparations and biological samples, including liquid chromatography<sup>8</sup> gas chromatography,<sup>9</sup> stripping voltammetry,

try,<sup>10</sup> electrogenerated chemiluminescence,<sup>11</sup> and fluorimetry.<sup>12</sup> Some of these methods are not suitable for routine analysis because they require sophisticated instruments that are not yet available in many laboratories. Some of the methods involve numerous steps and tedious processes, resulting in insufficient sensitivity. Spectrophotometric methods offer practical and economical advantages over other methods, providing sensitive and accurate results.<sup>13</sup>

Spectrophotometric methods for the determination of trimipramine are mainly based on coupling with a diazotized reagent, oxidative coupling of the drug with an electrophilic reagent,<sup>14</sup> ion-pair or charge-transfer complex formation,<sup>15</sup> or oxidation in an acidic medium using a suitable oxidizing reagent<sup>16</sup> for producing a colored product. These methods are usually multi-step, time consuming and sometimes involve a solvent extraction step with a poor concentration factor. On the other hand, the interference effects and spectrum complication due to the presence of complexation or oxidation reagents is also a major concern. In the present study, we report on the development of a simple, sensitive and interference free homogeneous liquid-liquid extractive-spectrophotometric method for the preconcentration and determination of trimipramine in pharmaceutical preparations and urine samples. A perfluorinated surfactant system is applied for homogeneous liquid-liquid extraction and preconcentration of trimipramine into a microdroplet sedimented phase. A simple modified spectrophotometric method, on the basis of trimipramine's reaction with strong nitric acid solution, is utilized for its direct determination in the resulting microdroplet.



Trimipramine Maleate (TPM)

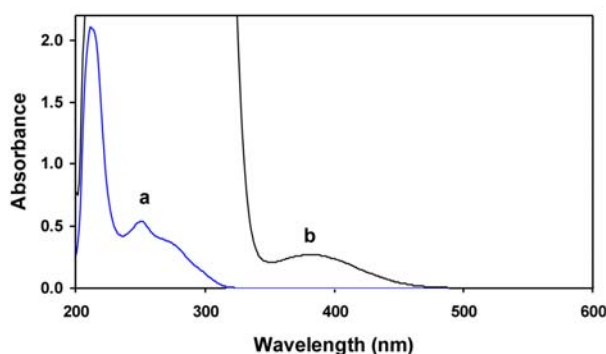
## 2. Results and Discussion

### 2. 1. Spectrophotometric Studies

Trimipramine reacts with concentrated acid solution at room temperature, resulting in a yellowish product. Farajzadeh et al.<sup>17</sup> stated that the yellow color arises from the oxidation of trimipramine. Some preliminary experiments with a complete literature survey revealed that probably there is no proof of a proper oxidation reaction on trimipramine which can transform it into a product with an absorption maximum in visible region. On the other hand, the color and spectrum of the yellowish product is in ac-

cordance with nitrobenzene spectrum. It should also be added that except nitric acid; other acids, such as HCl and H<sub>2</sub>SO<sub>4</sub>, and oxidizing agents, such as permanganate and hydrogen peroxide, can not produce a yellow color in trimipramine solution.<sup>17</sup> The yellow color in strong nitric acid solution may be due to nitration of benzene rings in para position relative to N group in cycloheptane ring.<sup>18</sup> The presence of NO<sub>2</sub> in the benzene rings induced a red shift in maximum absorption wavelength. The absorption spectra of TPM in pure water and aqueous nitric acid solutions are shown in Fig. 1. The spectra illustrate that the maximum absorbance of the yellowish product in acidic solutions occurs at 390 nm, whereas trimipramine does not show significant absorbance at this wavelength in pure water. The optimum concentration of nitric acid for the spectrophotometric determination of trimipramine in aqueous solution was found to be 2.2 mol L<sup>-1</sup>.

For direct determination of trimipramine in the sedimented liquid phase, resulting from homogeneous liquid-liquid extraction, a suitable organic solvent was needed. This solvent could not only dissolve the microdroplet easily but also should provide a proper medium for complete color development of trimipramine in its acidic solution. Different organic solvents, including methanol, ethanol, and acetone were examined. For this purpose, 10 mL solutions containing 50 µg TPM and 1.5 mL concentrated nitric acid using mentioned solvents were prepared and their UV-Vis spectra were recorded in the range of 200–800 nm. Acidic acetone solution of trimipramine produced a spectrum which wasn't suitable for quantitative purposes due to high absorption in the UV region. Trimipramine in ethanol acidic solution produced an unstable yellow color which gradually changed by time. However, trimipramine dissolved in acidic methanol got a stable yellow color with a distinct absorbance maximum at 390 nm which was stable at least for 24 h (the spectrum was similar to that of the aqueous solution of trimipramine as shown in Fig. 1). Hence, methanol was selected as the organic medium for dilution of sedimented liquid phase resulted from HLLE for subsequent spectrophotometric determination of trimipramine.



**Figure 1:** Absorption spectra for a 25 µg mL<sup>-1</sup> TPM sample in (a) water, (b) 1.5 mol L<sup>-1</sup> aqueous nitric acid solution.

Effect of the concentration of nitric acid in methanol for the determination of trimipramine in the sedimented liquid phase was also studied. As shown in Fig. 2, by increasing the concentration of nitric acid to  $6.5 \text{ mol L}^{-1}$ , the absorbance of the colored product increases and then remains constant. Thus,  $6.5 \text{ mol L}^{-1}$  was selected as optimal concentration of nitric acid in methanol for the spectrophotometric determination of trimipramine.

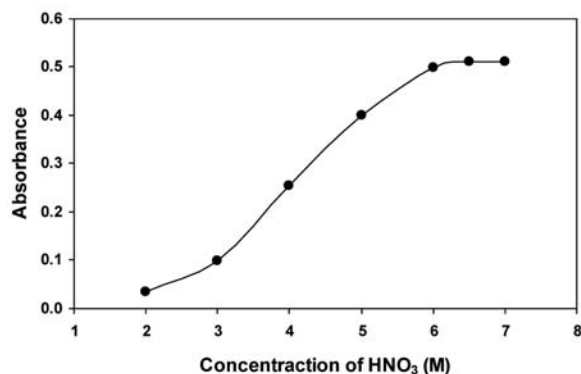
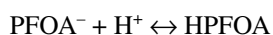


Figure 2: Effect of nitric acid concentration on the absorbance of TPM solution in methanol ( $25 \mu\text{g mL}^{-1}$ ).

## 2. 2. Development of the HLLE Method

The ternary component solvent system and the perfluorinated surfactant system are the two usual modes of HLLE. Different ternary component solvent systems were studied for HLLE of trimipramine. However, this system was not efficient due to incomplete phase separation after centrifugation. Thus, homogeneous liquid-liquid extraction using perfluorooctanoic acid (HPFOA) was examined.

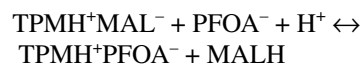
A fluorosurfactant such as HPFOA ( $\text{pK}_a = 1.01$ ,  $20^\circ\text{C}$ ,  $I = 0.1$  where  $I$  is ionic strength) dissolves in water at a pH value higher than its acid dissociation constant of the carboxylic acid group. If the pH of this aqueous solution is lower than its acid dissociation constant, the fluorosurfactant precipitates as a needle-like crystalline solid due to the charge neutralization of the carboxyl ion. However, if small amount of water-miscible organic solvent such as THF, acetone or acetonitrile coexists in this system, the fluorosurfactant precipitates in the water-miscible liquid phase with a  $\mu\text{L}$  volume scale. The solute is then extracted into the sedimented phase.<sup>19</sup> The reaction equation in this phase separation phenomenon and the acid dissociation constant ( $K_a$ ) are expressed as follows:



$$K_a = \frac{[\text{PFOA}^-][\text{H}^+]}{[\text{HPFOA}]}$$

On the other hand, lowering the pH by addition of acid protonates the maleate (counter ion of trimipramine) resulting in maleic acid in solution. Then  $\text{PFOA}^-$  can act as

counter ion of trimipramine (the acidic dissociation constant,  $K_a$ , of HPFOA is larger than maleic acid) and transfer it into organic phase, by charge neutralization and formation of a bulky lipophile ion pair, according to the following equation:



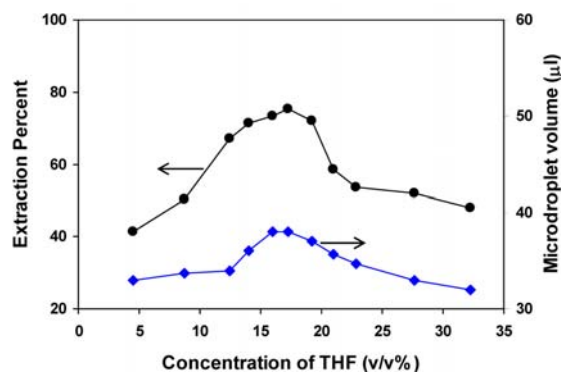
### 2. 2. 1. Effect of the Type and Concentration of Acid in Sample Matrix

To investigate the effect of type and concentration of acid in sample matrix on phase separation, volume of the sedimented phase and extraction efficiency of trimipramine,  $\text{HNO}_3$ ,  $\text{H}_2\text{SO}_4$ , and  $\text{HCl}$  were examined. Complete phase separation and higher extraction efficiencies were obtained using nitric acid. The concentration of nitric acid was also studied in the range of  $0.5$  to  $4 \text{ mol L}^{-1}$ . The highest extraction efficiency of trimipramine was observed in concentrations of  $1.0$  to  $1.2 \text{ mol L}^{-1}$  of nitric acid. Thus, further experiments were carried out using  $1.1 \text{ mol L}^{-1}$  of nitric acid as optimum amount. During the changes was made in the concentration of nitric acid in the sample matrix, significant differences were not seen in the volume of sedimented phase.

### 2. 2. 2. Effect of the Type and Concentration of the Water-miscible Organic Solvent

Acetone, ethanol, methanol, acetonitrile, THF, DMF and DMSO were tested as the water-miscible organic solvents for the homogeneous liquid-liquid extraction of trimipramine in the presence of HPFOA. Low extraction efficiency was obtained using DMF, methanol and acetone. The homogeneous solution undergoes a highly exothermic reaction after addition of concentrated nitric acid to trimipramine solution in ethanol. DMSO resulted in a suspension after centrifugation. The results show that the addition of THF resulted in a proper oily sedimented liquid phase and the highest trimipramine extraction efficiency. Furthermore, its application causes a complete phase-separation with the least amount of the solvent. Thus, THF was selected for subsequent experiments. The volume fraction of THF, in the initial homogeneous phase, was also studied in the range of  $4.5$ – $35\%$  (Fig. 3). The results showed that  $19\%$  THF was optimal for the generation of a proper initial homogeneous system and complete subsequent phase-separation with the highest extraction percents. On the other hand, this concentration provided a viscous spherical sedimented liquid phase, with a reasonable volume, suitable for handling with a micro-syringe. A delayed phase-separation was observed by using a THF volume fraction higher than  $20$ , while application of volume fractions lower than  $12$  produced a permanent homogeneous phase. Hence,  $19$  volume fraction was selected as

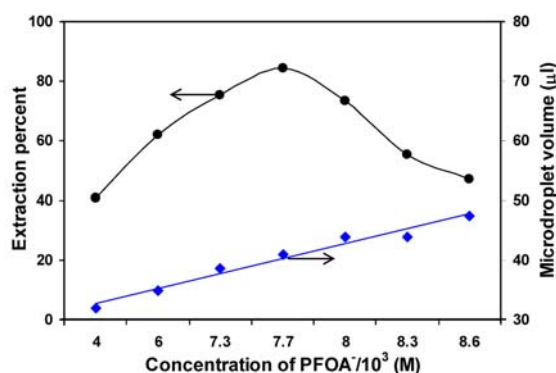
the optimal concentration of THF for further HLLE studies.



**Figure 3:** Effect of THF concentration on the extraction efficiency of trimipramine and volume of the microdroplet. Conditions: 25 µg of TPM in 13 mL of sample solution, Concentration of  $\text{HNO}_3 = 1.1 \text{ mol L}^{-1}$ .

### 2. 2. 3. Effect of the HPFOA Concentration

In order to investigate the optimum amount of HPFOA for the quantitative homogeneous liquid-liquid extraction of trimipramine, extraction of 25 µg of TPM from 13 mL of a sample solution under optimal experimental conditions was conducted by varying the concentration of HPFOA (Fig. 4). As the results show, the extraction of trimipramine increased with increasing of HPFOA concentration to  $7.7 \times 10^{-3} \text{ mol L}^{-1}$  and after that decreased with higher concentrations of HPFOA. The volume of the sedimented microdroplet showed a satisfactorily linear correlation with the concentration of HPFOA as reported in previous studies.<sup>20</sup> The subsequent HLLE experiments were carried out using  $7.7 \times 10^{-3} \text{ mol L}^{-1}$  of HPFOA as optimum amount.



**Figure 4:** Effect of HPFOA concentration on the extraction efficiency of trimipramine and volume of the microdroplet. Conditions: 25 µg of TPM in 13 mL of sample solution, Concentration of  $\text{HNO}_3 = 1.1 \text{ mol L}^{-1}$ , Concentration of THF = 19% (v/v).

The optimal centrifugation time and speed were also studied. The results showed that a centrifuge speed of

2500 rpm for greater than 15 min resulted in quantitative extraction. Hence, the optimum centrifugation condition (15 min at 2500 rpm) was used throughout this study.

### 2. 3. Analytical Performance

To investigate the linear range of the proposed HLLE method, sample solutions containing different concentrations of trimipramine was analyzed under the optimal experimental conditions. The results revealed that 0.01–7 µg TPM in 13 mL sample solution can be quantitatively extracted and determined using the proposed method. Application of the 13 mL sample solution produced a 36 µL sedimented liquid phase. Thus, the maximum concentration factor of the proposed method was determined to be 361-fold. The limit of detection (LOD) for TPM was determined from  $3\sigma$  (three times of standard deviation of blank sample) of 7 replicated measurements of the blank and division of the resulting value by the concentration factor and was found to be  $1.2 \text{ ng mL}^{-1}$ . The reproducibility of the proposed method for HLLE and the spectrophotometric determination of trimipramine were also studied. The results obtained for 10 replicate measurements of a sample solution containing  $1.92 \text{ µg mL}^{-1}$  of TPM resulted in a RSD of 2.9%.

### 2. 4. Application

To demonstrate the applicability of the proposed method to pharmaceutical preparations, it was used for the extraction and determination of trimipramine in tablets. The data in Table 1 indicate a satisfactory agreement between the results obtained by the proposed method and those reported by the official method.<sup>20</sup>

**Table 1:** Determination of trimipramine in pharmaceutical tablets<sup>a</sup>.

Sample	TPM (mg/tablets)	TPM determined (mg/tablets)	
		Proposed method	Official Method
A	25.0	$24.2 \pm 0.7$	$25.3 \pm 0.5$
B	25.0	$25.5 \pm 1.2$	$24.7 \pm 0.2$
C	25.0	$24.7 \pm 0.9$	$25.1 \pm 0.6$

<sup>a</sup> 13 mL of sample solution, Conditions: Concentration of  $\text{HNO}_3 = 1.1 \text{ mol L}^{-1}$ , Concentration of THF = 19% (v/v), Concentration of HPFOA =  $7.7 \times 10^{-3} \text{ mol L}^{-1}$ .

The proposed method was also applied for the determination of trimipramine in urine samples. Some preliminary analyses of spiked samples to demonstrate quantitative extraction of trimipramine in blank urine samples were done. It was revealed that the addition of 20 µg of TPM to 0.5 mL blank urine samples of A, B and C were recovered quantitatively with RSDs of 1.4%, 1.5% and 2.4%, respectively. Finally, the proposed method was used for the determination of trimipramine in the urine samples of three target persons 9, 12, and 24 h after consumption of

trimipramine (Table 2). A decrease in concentration with time is obvious as predicted.

**Table 2:** Analysis of trimipramine in urine samples<sup>a</sup>

Urine sample	Trimipramine determined ( $\mu\text{g mL}^{-1}$ )		
	After 9 h	After 12 h	After 24 h
Sample A	$4.4 \pm 0.3$	$2.1 \pm 0.3$	$0.9 \pm 0.1$
Sample B	$3.7 \pm 0.2$	$2.3 \pm 0.8$	$0.3 \pm 0.2$
Sample C	$4.2 \pm 0.5$	$1.9 \pm 0.5$	$1.2 \pm 0.5$

<sup>a</sup> 0.5 mL of urine sample in 13 mL total volume, Conditions: Concentration of  $\text{HNO}_3 = 1.1 \text{ mol L}^{-1}$ , Concentration of THF = 19% (v/v), Concentration of HPFOA =  $7.7 \times 10^{-3} \text{ mol L}^{-1}$ .

### 3. Conclusion

New and effective HLLE method was developed for preconcentration of trimipramine in a microdroplet sedimented liquid phase. A modified simple and interference free spectrophotometric method was used for the determination of trimipramine in the resulting microdroplet. Trace amounts of trimipramine in urine samples can be analyzed without any pretreatment process. The proposed method was successfully applied to the preconcentration and determination of trimipramine in pharmaceutical preparations and urine samples.

## 4. Experimental

### 4.1. Apparatus and Reagents

Absorbance measurements were carried out with a Shimadzu UV-1650PC double-beam spectrophotometer and a 200  $\mu\text{L}$  quartz cell at 390 nm as maximum absorption wavelength ( $\lambda_{\text{max}}$ ). An Eppendorf 5810 centrifuge was used for centrifugation.

Ethanol, methanol, dimethyl formamide (DMF), dimethyl sulfoxide (DMSO) and tetrahydrofuran (THF) were purchased in analytical reagent-grade from Merck or Fluka. All acids were of the highest purity available from Merck. Reagent-grade perfluorooctanoic acid (HPFOA) was purchased from Merck. Trimipramine maleate (TPM) was obtained from LundBeck Pharmaceutical Co. (Denmark). Doubly distilled deionized water was used for all analyses. A standard  $1000 \mu\text{g mL}^{-1}$  trimipramine stock solution was prepared by dissolving 0.1 g of trimipramine maleate in water and dilution to 100 mL. Standard working solutions were prepared from the stock solution. The stock and working solutions were stored at 4 °C.

### 4.2. Homogeneous Liquid-Liquid Extraction and Determination of Trimipramine

A sample solution containing  $0.01\text{--}7 \mu\text{g mL}^{-1}$  of trimipramine was placed in a 50.0 mL cylindrical teflon vial

fitted with a plastic cap. Then, 2.5 mL of THF and 2 mL of 0.05 M HPFOA solutions were added. The mixture was left to stand for 2 min at room temperature. Then, 1 mL of concentrated nitric acid was added (total volume fixed to 13 mL) and the mixture was centrifuged at 2500 rpm for 15 min. After an oily sedimented liquid phase was formed, its volume was determined using a 100  $\mu\text{L}$  microsyringe and transferred to a 0.2 mL spectrophotometric cell. After addition of 90  $\mu\text{L}$  of concentrated nitric acid, it was set to volume with methanol. The concentration of trimipramine was then determined at 390 nm against a reagent blank with an external linear calibration curve.

### 4.3. Determination of Trimipramine in Pharmaceutical Tablets

Ten tablets from each sample were weighed individually to obtain representative average weights. The tablets were ground into a fine powder. A 200 mg portion of the powder was transferred into 150 mL of 1:1 water-acetonitrile mixture and shaken vigorously followed by 5 min sonication using an ultrasonic bath for further dissolution. Filtration through a 0.45  $\mu\text{m}$  membrane filter (Millipore) was performed to remove any remaining insoluble matter. The membrane filter was then washed three times with the water-acetonitrile mixture. The filtrate and washing solutions were transferred into a 250 mL volumetric flask and diluted to the mark with distilled water. Finally a proper aliquot of the solution was analyzed using the proposed method discussed in previous section.

### 4.4. Determination of Trimipramine in Urine

Three healthy persons that had not consumed trimipramine lasting the month prior to the test were selected for the study. From each person a urine sample was taken before consumption of the drug for added-found analysis, and three samples 9, 12 and 24 h after the consumption. The samples were kept in a refrigerator until the final sample was collected. The proposed method allowed the detection of trimipramine in the collected urine samples without additional sample preparation steps. Rather, 0.5 mL of each urine sample was transferred to a 50 mL cylindrical centrifuge vial and subsequently analyzed using the proposed HLLE method.

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## Povzetek

Razvili smo enostavno, občutljivo in zanesljivo ekstrakcijsko spektrofotometrično metodo za določevanje trimipramina (TPM) v vzorcih urina. V močno kislem homogenem mediju je trimipramin tvoril obarvano spojino, ki smo jo ekstrahirali v sedimentirano mikrokapljico ob prisotnosti perfluorooktanoata (PFOA<sup>-</sup>). Koncentracijo ekstrahirane obarvane spojine v mikrokapljici smo določili z meritvijo absorbanca pri 390 nm po razredčenju z metanolom. Pri optimalnih eksperimentalnih pogojih ( $C(\text{HNO}_3) = 1,1 \text{ mol L}^{-1}$ ,  $C(\text{PFOA}^-) = 7,7 \cdot 10^{-3} \text{ mol L}^{-1}$ ,  $C(\text{THF}) = 19 \%$ ), smo lahko analizirali raztopine vzorcev, ki so vsebovale  $0,01 - 7 \mu\text{g mL}^{-1}$  TPM. Z ekstrakcijo v  $36 \mu\text{L}$  sedimentirane tekočine iz  $13 \text{ mL}$  raztopine vzorca smo dosegli koncentracijski faktor 361. Spodnja meja detekcije za opisano metodo je  $1,2 \text{ ng L}^{-1}$  in relativni standardni odklik  $2,9 \%$  za deset ponovitev pri določevanju  $1,92 \mu\text{g mL}^{-1}$  TPM. Predlagano metodo smo uspešno uporabili za ekstrakcijo in določevanje trimipramina v farmacevtskih preparatih in urinu. Rezultati so pokazali dobro ujemanje s certificiranimi analiznimi metodami.