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Short communication

# Determination of imipramine in presence of iminodibenzyl and in pharmaceutical dosage form

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**Abstract**

Two spectrophotometric methods for the determination of imipramine in presence of iminodibenzyl as an impurity are described. The first method is a ratio-spectra first derivative spectrophotometry, the signals were measured at 240.2 nm for imipramine. Calibration graph was found linear in the range 5–30 μg $ml^{-1}$. The second method is based on the reaction of imipramine base, being an electron donor, with *p*-chloranilic acid, being π acceptor, to form a purple colored charge transfer complex. The absorbance was measured at 520.5 nm without interference with iminodibenzyl. Both methods are rapid, simple and do not require any preliminary separation or treatment of the samples. Furthermore, the two methods were applied to pharmaceutical dosage form.


*Keywords:* Imipramine; Ratio-spectra derivative spectrophotometry; Iminodibenzyl; Pharmaceutical dosage form; *p*-Chloranilic acid; Spectrophotometry

## 1. Introduction

Imipramine is 10,11 dihydro-*N*,*N*-dimethyl-5H-dibenz[b,f]azepine-5-propanamine [1]. It is widely used for the treatment of depression and is often referred to as tricyclic antidepressant. Studies have indicated that the efficacy of this drug in alleviating depression at night is due to the enhancement of noradrenergic activity through the blockage of norepinephrine reuptake in blocking peripheral and central noradrenergic system [2].

Many analytical methods have been published for the determination of imipramine based on GC [3–6], HPLC [7–13], capillary electrophoresis [14,15], spectrophotometry [16,17], atomic absorption spectrometry [18,19], chemiluminescence [20] and electrochemical analysis [21,22].

In aqueous solution at pH 4, iminodibenzyl is the major by-product in heated samples [23].

There is a severe overlap in the absorption spectra of imipramine and iminodibenzyl, so here in this paper we present two methods for determination of imipramine in presence of its impurity iminodibenzyl without previous separation. The first of which is a ratio derivative spectrum for resolution of binary mixtures [24,25]. The second proposed method is a charge transfer method

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depending on basicity of imipramine. The two methods were applied successfully for the determination of imipramine in pharmaceutical formulation.

## 2. Experimental

### 2.1. Apparatus

A UV-1601 PC-UV visible Shimadzu spectrophotometer was used.

### 2.2. Samples

#### 2.2.1. Imipramine hydrochloride

This was obtained from Novartis Pharma, Egypt. It was analyzed by the British Pharmacopoeia (1998) method [26] and was found to contain $100.08 \pm 0.635$.

#### 2.2.2. Iminodibenzyl

It was purchased from Aldrich.

#### 2.2.3. Tofranil tablets 25 mg

This is a product of Novartis Pharma and was purchased from local pharmacies. Each tablet is labeled to contain 25 mg imipramine hydrochloride. Batch No. used was 172.

### 2.3. Stock solutions

#### 2.3.1. For p-chloranilic acid

*2.3.1.1. Imipramine working solution (1 mg ml$^{-1}$).* This was prepared by dissolving an amount of imipramine HCl equivalent to 100 mg of imipramine base in 30 ml distilled water, which was then transferred quantitatively to a 125 ml separating funnel. This solution was rendered alkaline with ammonia and extracted five times each with 20 ml dry chloroform.

The chloroformic extract was evaporated away from light and dissolved in acetonitrile. The solution was transferred to a 100 ml volumetric flask and completed to the mark with acetonitrile.

*2.3.1.2. Iminodibenzyl standard solution (1 mg ml$^{-1}$).* 100 mg iminodibenzyl were accurately weighed in a 100 ml volumetric flask, dissolved and completed to the mark with acetonitrile.

*2.3.1.3. Laboratory prepared mixtures.* Mixtures containing different ratios of imipramine and

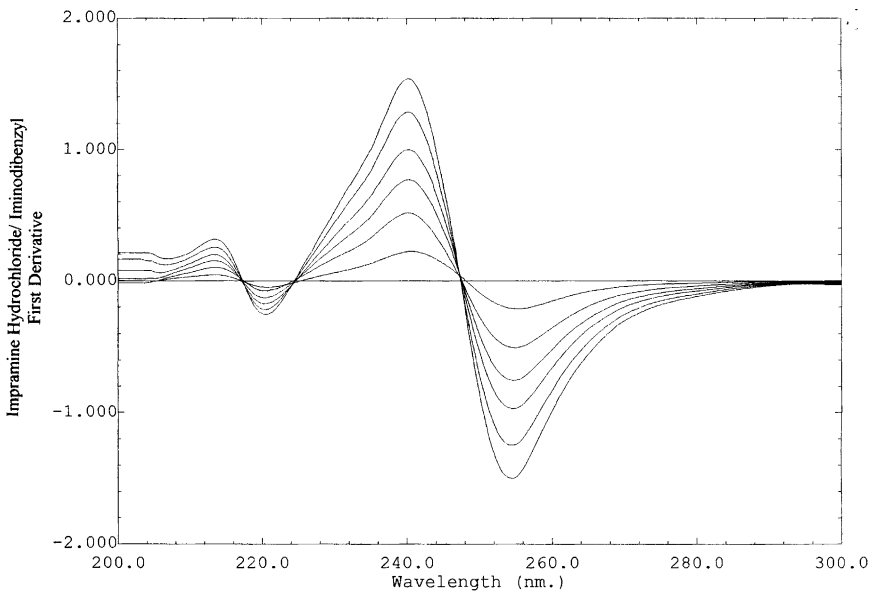


Fig. 1. First derivative of ratio spectra of imipramine using 3 μg ml$^{-1}$ iminodibenzyl as a divisor.

iminodibenzyl were prepared. They are prepared to contain 10–90% of iminodibenzyl as an impurity.

*2.3.2. For ratio spectra derivative*

*2.3.2.1. Imipramine hydrochloride standard solution (1 mg $ml^{-1}$).* 0.1 g of imipramine hydrochloride was weighed in a 100 ml volumetric flask, dissolved in methanol and the volume was completed to the mark with methanol.

*2.3.2.2. Iminodibenzyl standard solution (1 mg $ml^{-1}$).* 0.1 g of iminodibenzyl was dissolved in methanol in a 100 ml volumetric flask. The volume was then completed to the mark with methanol.

*2.3.2.3. Imipramine hydrochloride working solution (0.1 mg $ml^{-1}$).* 10 ml of imipramine HCl standard solution were further diluted by transferring to a 100 ml volumetric flask and completing the volume to the mark with methanol.

*2.3.2.4. Iminodibenzyl working solution (0.1 mg $ml^{-1}$).* 10 ml of iminodibenzyl standard solution were transferred to a 100 ml volumetric flask and diluted to the mark with methanol.

*2.3.2.5. Laboratory prepared mixtures.* Mixtures containing different ratios of imipramine and its impurity iminodibenzyl were prepared to contain 10–60% of the impurity.

*2.4. Reagents and solvents*

*p*-Chloranilic acid (Sigma, product of Austria) was freshly prepared in a concentration of 0.1% w/v in acetonitrile (Fisons, England). Chloroform and ammonia used were purchased from El-Nasr Company. Methanol used for ratio derivative spectrum was obtained from Riedel-deHaën, Sigma-Aldrich Laborchemikalien GmbH, Germany.

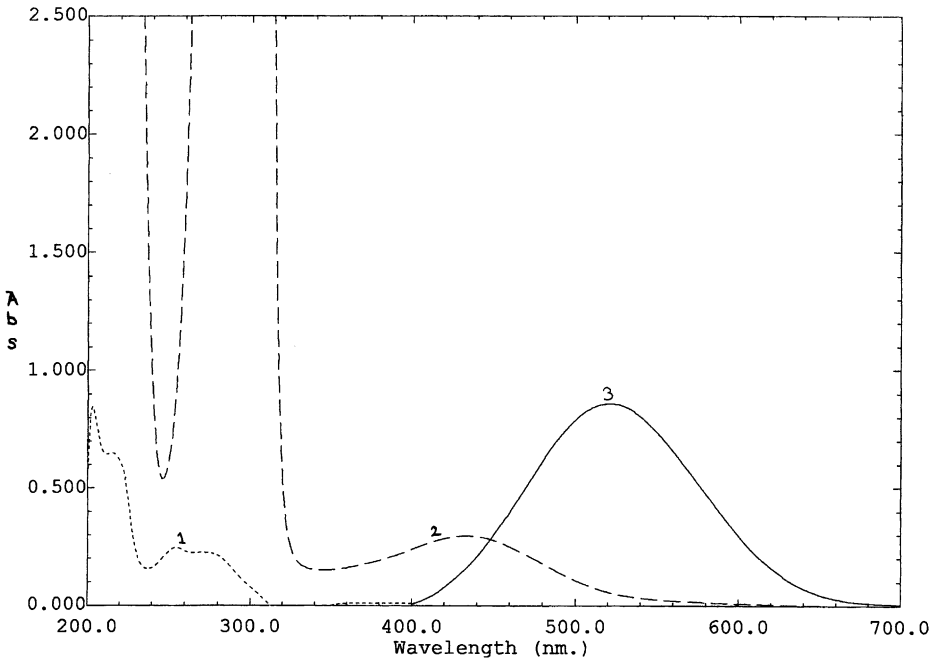


Fig. 2. Absorption spectra of imipramine (1), *p*-chloranilic acid (2), against acetonitrile blank, and imipramine–*p*-chloranilic acid complex against a reagent blank.

## 3. Procedure

### 3.1. Calibration

#### 3.1.1. For p-chloranilic acid

Different portions of imipramine working solution (0.2–2 ml), were transferred into a series of 10 ml volumetric flasks, 3 ml 0.1% w/v *p*-chloranilic acid were added and the volume was completed to the mark with acetonitrile. The contents were mixed and absorbance of each solution was measured at 520.2 nm against a reagent blank.

#### 3.1.2. For first derivative of ratio spectra

Aliquots of imipramine HCl working solution (0.5–3 ml) were transferred into a series of 10 ml volumetric flasks. The volume was completed to the mark with methanol. Absorption spectra were recorded and divided by the spectrum of 3 ug $ml^{-1}$ of iminodibenzyl. All spectra resulting from that division were stored on the IBM-PC and their first derivatives were computed with $\Delta\lambda = 4$ nm intervals (Fig. 1). The concentration of imipramine was determined by measuring the amplitude of the signals at 240.2 nm. Then the amplitude of the signal at 240.2 nm was plotted against the corresponding concentration to construct the calibration curve.

### 3.2. Analysis of pharmaceutical dosage form

#### 3.2.1. Analysis of tablets by charge transfer method

The average weight of tablet was determined, and then 20 tablets were finely powdered in a mortar. A quantity of powdered tablet equivalent to 100 mg imipramine base were accurately weighed and transferred to a beaker. About 40 ml distilled water were added and solution was shaken mechanically using a magnetic stirrer for 30 min. The solution was then filtered and transferred quantitatively to a 125 ml separating funnel, made just alkaline by ammonia and extracted five times each with 20 ml dry chloroform. The chloroformic extract was left to evaporate away from light and the residue was dissolved in acetonitrile then transferred quantitatively to a 100 ml volumetric flask. The volume was completed to the mark with acetonitrile. The method in Section 3.1.1 was adopted to the prepared solution.

#### 3.2.2. Analysis of imipramine tablets by using first derivative of ratio spectra

20 tablets of Tofranil were powdered and mixed well. A quantity of the powdered tablets equivalent to 100 mg of imipramine HCl was accurately weighed into a 100 ml volumetric flask. Methanol was added to the mark. The flask was shaken mechanically for 15 min using a magnetic stirrer. The solution was then filtered into a dry conical flask and 10 ml of this solution were transferred into a 100 ml volumetric flask and the volume was completed to the mark with methanol. The final solution (0.1 mg $ml^{-1}$) was then determined as in Section 3.1.2.

### 3.3. Laboratory prepared mixtures

#### 3.3.1. For p-chloranilic acid method

The working solution of imipramine base was mixed with iminodibenzyl standard solution in different ratios within the range 20–200 μg $ml^{-1}$. Same steps were adopted as in Section 3.1.1 and absorbance was measured at 520.5 nm using a reagent blank.

#### 3.3.2. For first derivative of ratio spectra

The working solution of imipramine HCl was mixed with its impurity in different ratios within the range 5–30 μg $ml^{-1}$. The solution was treated as in Section 3.1.2 and the signal was measured at 240.2 nm.

## 4. Results and discussion

### 4.1. For p-chloranilic acid method

#### 4.1.1. Reaction involved

A purple color was instantaneously produced when *p*-chloranilic acid solution was added to imipramine solution in acetonitrile. The $\lambda_{max}$ of the formed complex was at 520.2 nm (Fig. 2). The change in color of the mixture of imipramine and *p*-chloranilic acid indicate charge transfer complex formation between the two compounds. This

Table 1
Special characteristics for the calibration graphs of imipramine resulting from the proposed derivative ratio and charge transfer complex methods

| Parameter | Derivative ratio | P-CA |
|---|---|---|
| Linear range (μg $ml^{-1}$) | 5–30 | 20–200 |
| $\lambda_{max}$ | 240.2 nm | 520.5 nm |
| LOD [28] | 0.14 | 4.7 μg $ml^{-1}$ |
| LOQ [28] | 0.44 | 14.1 μg $ml^{-1}$ |
| Accuracy | 99.98 ± 0.773 | 100.08 ± 0.724 |
| Precision | 100.33 ± 0.320 | 99.98 ± 1.027 |
| Intercept (b) | − 0.0229 | 0.0078 |
| R.S.D. of Intercept | 0.1639 | 0.0498 |
| Slope ($a$) | 0.0521 | 0.0043 |
| R.S.D. of Slope | 0.0183 | 0.0012 |
| Correlation coefficient ($r$) | 0.99996 | 0.99997 |
| Regression equation | $P = 0.0521C -$ 0.0229 | $A = 0.0043C +$ 0.0078 |
| $\varepsilon$ | | $1.2 \times 10^3$ |

Where $P$ is the peak amplitude and $A$ is the absorbance of the complex formed.

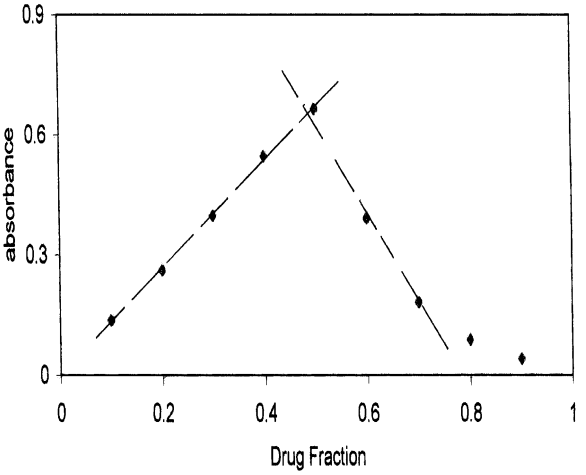


Fig. 3. Continuous variation method applied for $p$-chloranilic acid and imipramine.

complex is probably formed through the lone pair of electron donated by imipramine (n-donor) to $p$-chloranilic acid (π-acceptor) in which a partial ionic bond is assumed to be formed [27]. On the other hand iminodibenzyl which is an impurity of imipramine and also is one of its degradation products [23], did not produce any color with $p$-chloranilic acid. So, we took advantage of this for the determination of imipramine in presence of iminodibenzyl without any interference.

### 4.1.2. Effect of reaction variables

*4.1.2.1. Effect of volume of reagent solution.* It was found that 3 ml 0.1% $p$-chloranilic acid solution in acetonitrile is sufficient for the reaction to occur.

*4.1.2.2. Solvent choice.* Several solvents such as methanol, ethanol, chloroform, dioxane, acetone and acetonitrile were tried. Acetonitrile was found to give the highest absorbance and also has the advantage of being a suitable solvent for the drug and the reagent.

Table 2
Analysis of pure imipramine samples using the proposed derivative ratio and charge transfer methods

| Derivative ratio | | | P-CA | | |
|---|---|---|---|---|---|
| Taken (μg $ml^{-1}$) | Found (μg $ml^{-1}$) | Recovery (%) | Taken (μg $ml^{-1}$) | Found (μg $ml^{-1}$) | Recovery (%) |
| 5 | 4.95 | 99.00 | 20 | 20.04 | 100.20 |
| 10 | 10.11 | 101.11 | 40 | 40.51 | 101.28 |
| 15 | 14.89 | 99.27 | 70 | 69.22 | 98.89 |
| 20 | 20.06 | 100.30 | 100 | 99.80 | 99.80 |
| 25 | 25.07 | 100.28 | 130 | 130.62 | 100.48 |
| 30 | 29.96 | 99.87 | 160 | 159.80 | 99.88 |
| Mean ± R.S.D. | | 99.98 ± 0.773 | 200 | 200.04 | 100.02 |
| | | | Mean ± R.S.D. | | 100.08 ± 0.724 |

Table 3
Standard addition technique for the determination of imipramine by derivative ratio and P-CA methods

| Derivative ratio | | | | P-CA | | | |
|---|---|---|---|---|---|---|---|
| Taken (μg ml$^{-1}$) | Authentic added (μg ml$^{-1}$) | Authentic found (μg ml$^{-1}$) | Recovery (%) | Taken (μg ml$^{-1}$) | Authentic added (μg ml$^{-1}$) | Authentic found (μg ml$^{-1}$) | Recovery (%) |
| 5 | | 5.04 | 100.73 | 25 | | 24.74 | 98.97 |
| 5 | 5 | 4.99 | 99.80 | 25 | 40 | 40.27 | 100.68 |
| 5 | 10 | 10.00 | 100.00 | 25 | 70 | 70.39 | 100.56 |
| 5 | 15 | 15.92 | 100.47 | 25 | 100 | 99.33 | 99.33 |
| 5 | 20 | 19.92 | 99.60 | 25 | 130 | 129.45 | 99.58 |
| 5 | 25 | 25.22 | 100.88 | 25 | 160 | 160.74 | 100.46 |
| Mean ± R.S.D. | | | 100.15 ± 0.520 | Mean ± R.S.D. | | | 100.12 ± 0.619 |

*4.1.2.3. Effect of time on the reaction and stability of the formed complex.* The reaction between the drug and the reagent was found to be instantaneous; therefore, the absorbance was measured directly without standing. Furthermore, the complex formed was found to be stable for over 4 h.

*4.1.2.4. Assessment of the stoichiometry of the reaction.* In order to ascertain the stoichiometry of the reaction, Job's method of continuous variation was applied. Solutions of $1.1 \times 10^{-3}$ M of both imipramine and *p*-chloranilic acid were prepared. The results revealed that *p*-chloranilic acid react with imipramine in a ratio 1:1 (Fig. 3).

*4.1.3. Quantification*

A linear correlation was obtained between absorbance at 520.5 nm and concentration of the drug. Linearity was achieved in a concentration range of 20–200 μg ml$^{-1}$ as given in Table 1.

Correlation coefficient, molar absorbitivity, detection limit, intercept, slope and regression equation for the calibration data of the drug are also given. Mean percentage recovery was found to be 100.08 ± 0.724 at 520.5 nm as shown in Table 2.

*4.2. Quantification of imipramine by using first derivative of ratio spectra*

Upon plotting a graph relating the amplitude of the signal of the first derivative of ratio spectra at 240.5 nm and the concentration, linearity was attained in the range 5–30 μg ml$^{-1}$. Correlation coefficient, intercept, slope and regression equation for the calibration data of the drug are given in Table 1.

Concentration of imipramine may be determined either by using the regression equation or from the graph. Mean percentage recovery was found to be 99.98 ± 0.773 at 240.5 nm as shown in Table 2.

*4.3. Reproducibility, precision and validity of the two proposed methods*

Reproducibility of results obtained by analysis of the sample at different times was determined and satisfactory results were obtained.

Table 4
Determination of imipramine by derivative ratio and charge transfer methods in laboratory prepared mixtures in presence of iminodibenzyl

| Derivative ratio | | P-CA | |
|---|---|---|---|
| Iminodibenzyl (%) | Intact imipramine recovery (%) | Iminodibenzyl (%) | Intact imipramine recovery (%) |
| 10 | 100.67 | 10 | 99.37 |
| 20 | 101.08 | 20 | 99.86 |
| 30 | 99.90 | 30 | 99.94 |
| 40 | 101.39 | 40 | 99.17 |
| 50 | 101.53 | 50 | 99.91 |
| | | 60 | 100.06 |
| | | 70 | 99.85 |
| | | 80 | 101.85 |
| | | 90 | 100.20 |
| Mean ± R.S.D. | 100.91 ± 0.650 | Mean ± R.S.D. | 100.02 ± 0.758 |

Table 5
Statistical comparison between the results obtained by applying the proposed derivative ratio and P-CA and those obtained by applying the official method for the analysis of pure imipramine

| Item | Derivative ratio | P-CA | Official B.P. (1998) method |
|---|---|---|---|
| Mean | 99.98 | 100.08 | 100.10 |
| S.D. | 0.773 | 0.725 | 0.652 |
| *N* | 6 | 7 | 7 |
| Variance | 0.598 | 0.526 | 0.425 |
| Student's *t*-test | 0.304 (2.201) | 0.050 (2.179) | |
| *F*-value | 1.41 (4.95) | 1.24 (4.28) | |

Figures in parentheses are the corresponding theoretical *t*- and *F*-values ($P = 0.05$) [29].

Replicate determination of different concentrations was carried out. The concentrations were then calculated from the respective regression equations. The results were summarized in Table 2.

The proposed methods have been successfully applied to tablets containing imipramine. Applying standard addition technique further assessed the results (Table 3)

Results of the proposed procedures were statistically compared with those obtained by reported methods [26]. Table 5 shows that the calculated $t$ and $F$ values are less than the theoretical ones, indicating no significant differences between the proposed procedures and the reported ones.

### 4.4. Determination of imipramine in presence of its impurity iminodibenzyl

Iminodibenzyl is one of the degradation products of imipramine and also may be present in imipramine powder as a synthetic impurity [23].

The proposed procedures were used to determine the intact drug in presence of its impurity. Iminodibenzyl was found not to react with *p*-chloranilic acid reagent.

Table 4 shows the results obtained upon analysis of synthetic mixtures containing intact drug and impurity in different ratios. It was found that the proposed *p*-chloranilic acid method could be successfully used for the determination of intact drug in presence of up to 90% of its impurity

iminodibenzyl. While in case of first derivative of ratio spectra imipramine HCl can be selectively determined in presence of up to 50% iminodibenzyl.

## 5. Conclusion

*p*-Chloranilic acid method and first derivative of ratio spectra permit simple, rapid and direct determination of imipramine in presence of iminodibenzyl without preliminary separation. These methods have the advantage over HPLC and GC of speed and reduced cost without losing accuracy and precision.

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