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Journal of Pharmaceutical and Biomedical Analysis
22 (2000) 189–196

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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# Spectrophotometry assay of imipramine and desipramine using ammonium metavanadate and its application to pharmaceutical preparations

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Received 21 June 1999; received in revised form 22 October 1999; accepted 8 November 1999

**Abstract**

Simple and sensitive method for determination of imipramine and desipramine is reported. The procedure is based on the oxidation of the drugs by ammonium metavanadate. Linear calibration graphs were obtained in the concentration range 0.6–40 μg $ml^{-1}$ of imipramine and 0.7–35 μg $ml^{-1}$ of desipramine with a relative standard deviation (RSD) less than 0.5%. The method was applied to the determination of the drugs in pharmaceutical preparations and compared favourably with independent official methods. 

*Keywords:* Spectrophotometric; Imipramine hydrochloride; Desipramine hydrochloride; Pharmaceutical preparations

## 1. Introduction

Dibenzoazepine derivatives are widely used among the tricyclic antidepressants. Their wide application in medicine requires methods for their determination in body fluids or in pharmaceuticals. Among the methods adopted for the determination of dibenzoazepines are chromatographic [1–4], electrochemical [5–8], spectrophotometric [9–11], spectrofluorimetric [12] and others [13–15]. The official methods normally involve titrations in non-aqueous medium or a spectrophotometric procedure [16,17].

A variety of HPLC methods are now widely used in routine application owing to their sensitivity, specificity and low cost [18,19]. The methods have adequate sensitivity in order to quantify low concentrations of the drugs in biological fluids. Use of these methods is justified when sample matrix is rather complex and the dibenzoazepine concentration low, as is usually the case with clinical samples. However, in pharmaceutical analysis, where the sample matrix is usually less complex and analyte concentration levels are fairly high, the main aim is to develop fast, simple, inexpensive methods, that can readily be adapted for routine analysis at relatively low cost to the different requirements of analytical problems.

Although immunochemical techniques [20,21] seem to be, in general terms more sensitive, they have many disadvantages — the larger, the num-

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PII: S0731-7085(99)00287-3

ber of steps in procedure, the longer is the time consumed to carry out the assays.

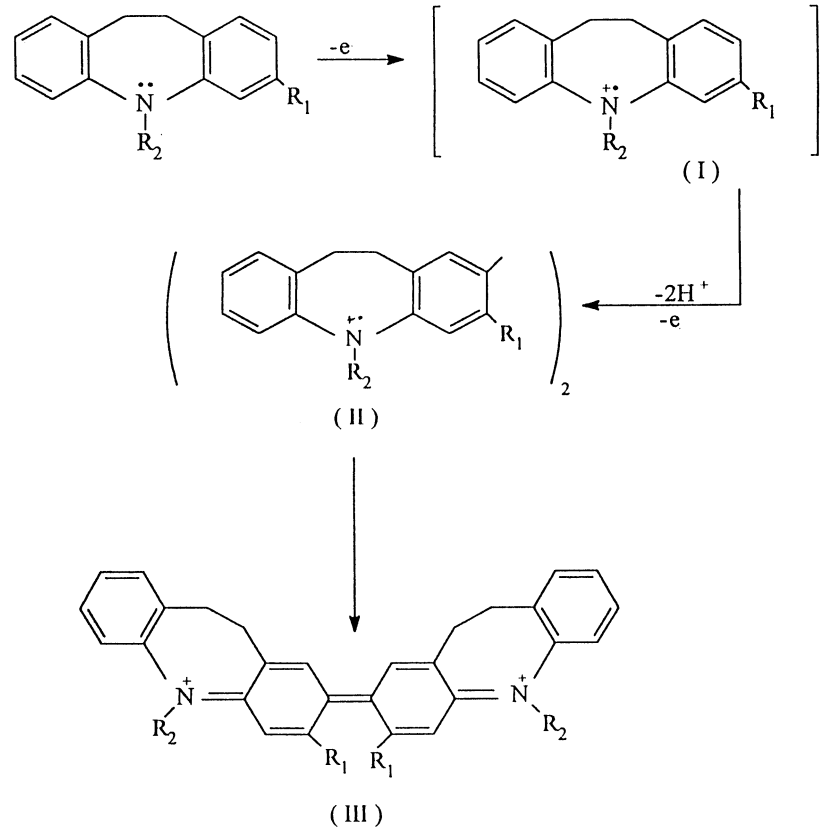
The number of spectrophotometric methods of dibenzoazepine determinations is very limited but spectrophotometry, due to its simplicity is by far the most popular method of analysis, which finds wide application in various fields of routine analysis, including pharmaceuticals.

Review of the methods for determination of dibenzoazepine derivatives has recently been presented by Mohamad et al. [22].

On the other hand, dibenzoazepines, due to their characteristic structure (the presence of chemically active nitrogen atoms) exhibit a number of interesting analytical properties [22].They react with halide and thiocyanate complexes of metals, e.g. Fe(III), Ti(IV), Nb(V), Cr(III), Zn(II) and with organic substances, e.g. picric acid, pyrocatechol violet, chromeazurol S to form coloured ion-association compounds.

They also form blue oxidation products with different oxidants, e.g. $Ce(SO_4)_2$, $K_2Cr_2O_7$, $KIO_4$, $(NH_4)_2S_2O_8$, $NH_4VO_3$. According to Renfroe et al. [23], mechanism of reaction of the oxidation of dibenzoazepine derivatives can be expressed as follows in Scheme 1.

The first step in the process was postulated to be the formation of cation radical (**I**). Fast coupling of (**I**) at position 2 leads to form a dimeric compound (**II**). It was suggested to be the next step of the process. The final oxidation of (**II**) gave the intensive coloured blue dimeric species (**III**).




Scheme 1. The oxidation of dibenzoazepines.

The mentioned properties have been exploited in the development of spectrophotometric methods of determination of dibenzoazepines. Recently Horria et al. [24] have proposed a method based on the oxidation of some dibenzoazepines with potassium dichromate in medium of sulphuric acid at temperature of 50°C heating for 25 min. Another procedure was based on oxidation at 75°C by iron(III) ions in $CH_3COOH$ medium [25]. Puzanowska–Tarasiewicz et al. [26] have used cerium(IV) sulphate(VI) and potassium iodate(VII) as oxidising agents. Karpińska et al. [25] have also applied pyrocatechol violet forming with imipramine hydrochloride (IMI) ion pair compound quantitatively extracted into mixture of chloroform–butyl alcohol (2:1, v/v).

Most of proposed oxidants for spectrophotometric determination of dibenzoazepines are unsatisfactory for different reasons, e.g. some of them require heating, long time for maximum colour development, lengthy procedure, narrow ranges of determinations or lack sensitivity and specificity.

As a continuation of our research according tricyclic psychotropic drugs [27–31] this paper describes a simple and sensitive spectrophotometric method for the determination of imipramine and desipramine as representative compounds of dibenzoazepines. The method is based on the oxidation of the drugs by ammonium metavanadate. Ammonium metavanadate was used as oxidant to yield coloured blue oxidised stable products and the reactions equilibrium state were obtained very quickly.

Preliminary investigations revealed that the coloured products were more stable at room temperature than that obtained when iron(III) ions, potassium dichromate or iodate(VII) were used; this will encourage the use of ammonium metavanadate for better reproducible results. Application of the easily accessible cheap oxidant and less drastic reaction conditions makes it possible to increase the sensitivity of reactions and decrease cost of azaphenothiazine determinations.

The proposed method has been successfully applied to the assay of these drugs in pharmaceuticals.

## 2. Experimental

### 2.1. Reagents

A stock solution of IMI or desipramine hydrochloride (DES) from Sigma Chemical Co (both) was prepared by dissolving the requisite amount of sample in distilled water. The solution was prepared fresh every day and kept in the dark and cold to minimise oxidation.

Standard ammonium metavanadate ($NH_4VO_3$) from Fluka (Switzerland) solution $1 \times 10^{-2}$ mol $l^{-1}$ was prepared by dissolving 0.5850 g of sample in the appropriate concentration of sulphuric acid solution and made up to volume with the same acid solution in a 500 ml calibrated flask.

Working solutions were prepared by dissolving the appropriate stock solutions.

All chemicals used were of analytical grade.

### 2.2. Apparatus

A Spekol 11 spectrophotometer (Carl Zeiss, Jena, Germany) was used for absorbance measurements. A Hewlett Packard Model 8452 A diode-array spectrophotometer was used for spectral analysis of the coloured compounds.

### 2.3. General procedure for determination of imipramine and desipramine

In a 10 ml volumetric flask was placed 1 ml of $4 \times 10^{-3}$ mol $l^{-1}$ solution of $NH_4VO_3$, 6 ml of 10 mol $l^{-1}$ sulphuric acid, 1 ml of $4 \times 10^{-4}$ mol $l^{-1}$ imipramine or desipramine and is made up to 10 ml with distilled water. The intensive coloured blue products formed were stable for 2 h. The absorbance was measured after 1 min at $\lambda = 620$ and 618 nm, respectively, against a reagent blank.

#### 2.3.1. Tablets analysis

The declared concentration of imipramine or desipramine was 10 or 25 mg, respectively. Twenty tablets of each drug were powdered and thoroughly mixed. An accurately weighted portion of the powder, equivalent to 10 mg of IMI or 25 mg of DES was transferred into 100 ml calibrated flask and made up to the mark with dis-

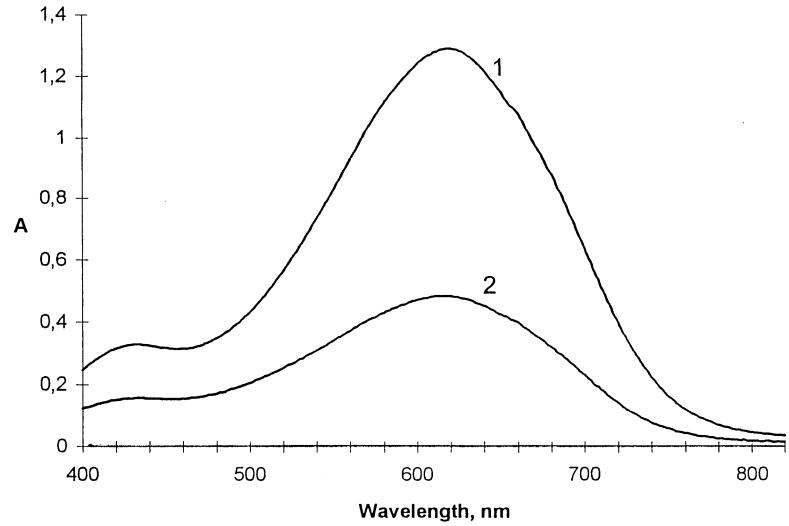



Fig. 1. Absorption spectra in VIS region. 1, Coloured product of IMI with $NH_4VO_3$; 2, coloured product of DES with $NH_4VO_3$; $C_{IMI} = 10^{-4}$ mol $l^{-1}$; $C_{DES} = 2.5 \times 10^{-5}$ mol $l^{-1}$; $C_{NH_4VO_3} = 2 \times 10^{-4}$ mol $l^{-1}$.

tilled water. The dibenzoazepine content in the diluted solution was determined as described above using the recommended spectrophotometric procedure.

*2.3.2. Injection analysis*

The declared concentration of imipramine in injection Imipramin was 25 mg in 2 ml of solution and ten different samples were analysed. The appropriate volume of sample was transferred into a 100 ml calibrated flask and diluted with distilled water. The IMI content was determined by the described spectrophotometric procedure.

## 3. Results and discussion

Small amounts of imipramine or desipramine react with an excess of ammonium metavanadate to form soluble intensely coloured products. Maximum intensity of the colour was obtained instantaneously at room temperature in acid solutions in the optimal concentrations at 5–8 mol $l^{-1}$ $H_2SO_4$ in the IMI–$NH_4VO_3$ and DES–$NH_4VO_3$ systems and remained constant for 2 h. Analytical measurements were made in sulphuric acid medium at concentration of 6 mol $l^{-1}$.

The absorption spectra of ammonium metavanadate and its coloured products with imipramine and desipramine are shown in Fig. 1. The $\lambda_{max}$ of coloured products were experimentally found to be 620 and 618 nm for IMI–$NH_4VO_3$ and DES–$NH_4VO_3$ systems, respectively.

Vanadium(IV) produced from the reduction of metavanadate under the used reaction conditions shows a maximum absorption peak at 740 nm. Determinations were carried out at 620 or 618 nm as it is the most intense peak and far from interferences due to reduction of metavanadate anion.

The coloured oxidation products, similar to dibenzoazepines also exhibit maximum absorption in UV region and they are given in Fig. 2. The UV spectra of the examined products of IMI or DES with $NH_4VO_3$ show maximum absorption at the same $\lambda = 194$ nm.

The effect of changes in the concentration of ammonium metavanadate was studied by measuring the absorbance for solutions containing a fixed amount ($4 \times 10^{-5}$ mol $l^{-1}$) of imipramine or desipramine and various amounts of ammonium metavanadate. The results are given in Fig. 3. The rate of formation and the colour intensity

of the products increased with increasing concentration of ammonium metavanadate, but constant and maximum absorbance readings were obtained for the eight fold excess of $NH_4VO_3$ with respect to imipramine or desipramine. The use of even higher fold excess of oxidant did not further influence the production and absorbance of the oxidation products.

### 3.1. Optimisation of variables

In order to optimise the proposed method, the influence of various experimental parameters on the optimal conditions of oxidation and reproducibility of the results was examined. It was known, from preliminary studies that the oxidation process of the investigated compounds was

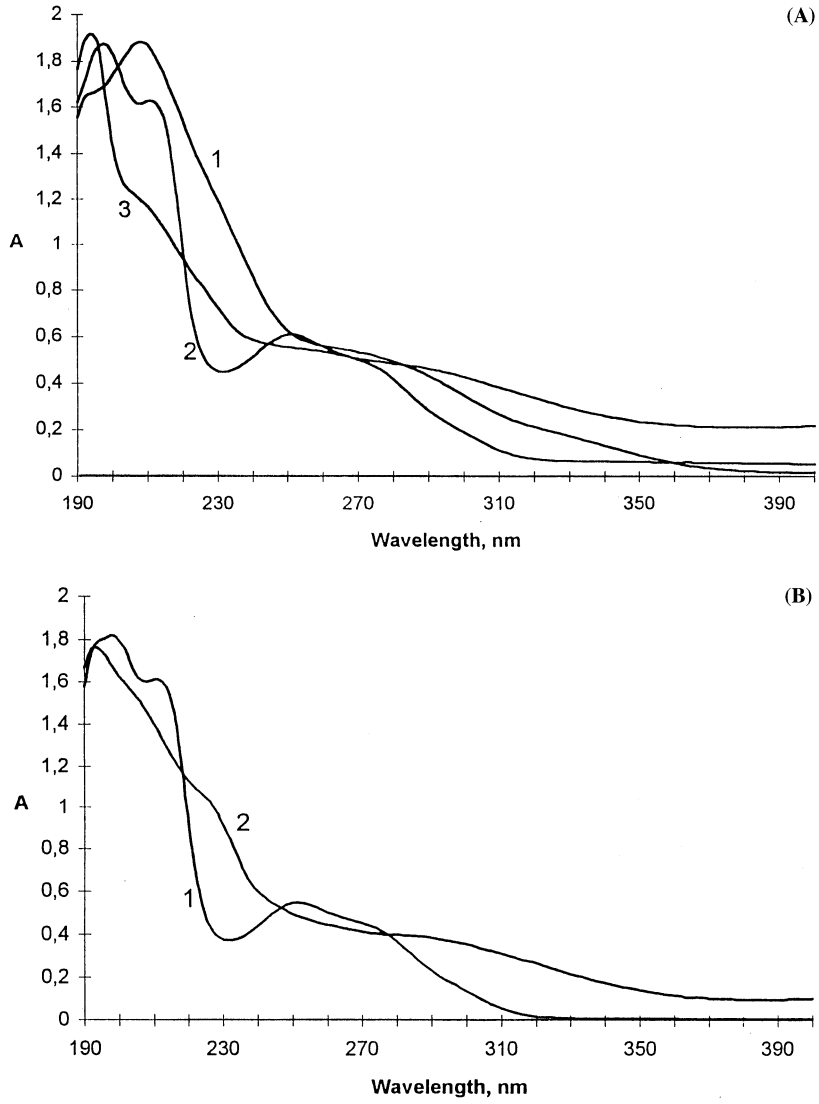



Fig. 2. Absorption spectra in UV region in IMI–$NH_4VO_3$ (A) and DES–$NH_4VO_3$ (B) systems. (A) 1, water solution of $NH_4VO_3$, $C = 7 \times 10^{-4}$ mol $l^{-1}$; 2, water solution of IMI, $C = 6 \times 10^{-5}$ mol $l^{-1}$; 3, coloured product of IMI oxidation, $C_{IMI} = 2.5 \times 10^{-5}$ mol $l^{-1}$, $C_{NH_4VO_3} = 1.25 \times 10^{-4}$ mol $l^{-1}$. (B) 1, water solution of DES, $C = 7 \times 10^{-5}$ mol $l^{-1}$; 2, coloured product of DES oxidation, $C_{DES} = 2.5 \times 10^{-5}$ mol $l^{-1}$, $C_{NH_4VO_3} = 1.25 \times 10^{-4}$ mol $l^{-1}$.

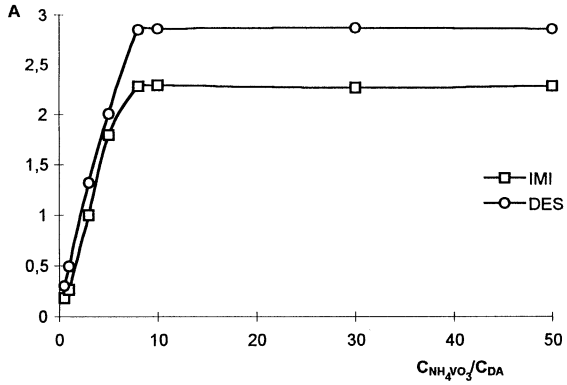



Fig. 3. Effect of the excess of ammonium metavanadate on absorbance of coloured product of imipramine (1) and desipramine (2).

strongly dependent on

- concentration and type of oxidant and acid,
- time and temperature of reaction.

*3.1.1. Influence of oxidants*

It was found that the oxidation reaction is strongly influenced by the type and concentration of oxidant. The effect of type of oxidant appeared in the kinetics of the reaction and in spectral characteristics of the coloured reaction products. The maximum of reaction product absorbance occurred at 620 nm in IMI–$NH_4VO_3$ system and at 618 nm in DES–$NH_4VO_3$ system. It was observed that by using an oxidant like $NH_4VO_3$ the colour of the product developed faster and required lesser concentrations of oxidant. The effect of ammonium metavanadate was studied in the range $3 \times 10^{-4}$–$4 \times 10^{-3}$ mol $l^{-1}$ in IMI–$NH_4VO_3$ and DES–$NH_4VO_3$ systems. A $4 \times 10^{-4}$ mol $l^{-1}$ concentration was selected for further study as optimal.

*3.1.2. Effect of type and concentration of acids*

The effect of the concentration of acids ($H_2SO_4$, $H_3PO_4$, $HClO_4$, HCl) on colour intensity was studied for each system. It was observed that the influence of HCl or $CH_3COOH$ on the absorbance produced in the IMI–$NH_4VO_3$ or DES–$NH_4VO_3$ is negligible. The observed intensity of colour was weak in $CH_3COOH$ or unstable in HCl. A significant increase in intensity and stability of absorbance was observed in the presence of $H_2SO_4$, $H_3PO_4$ or $HClO_4$ in the reaction solution. A maximum and stable absorbance was obtained when $H_2SO_4$ solutions were used at concentration range 5–8 mol $l^{-1}$. The 6 mol $l^{-1}$ solution of $H_2SO_4$ was used for further investigation of IMI–$NH_4VO_3$ and DES–$NH_4VO_3$ systems.

*3.1.3. Influence of temperature*

It was observed that the oxidation product of imipramine and desipramine formed immediately when $NH_4VO_3$ was used as oxidant. The intensity and stability of coloured products for further study of imipramine–$NH_4VO_3$ and desipramine–$NH_4VO_3$ were maximum at room temperature. No effect was noticed from the increase of reaction temperature up to 40°C for 30 min.

*3.2. Analytical evaluation*

Ammonium metavanadate has been tested as reagent for spectrophotometric determination of IMI and DES.

Typical calibration graphs for determination of the drugs obtained under the optimised conditions and from these results were linear over the range 0.6–40 μg $ml^{-1}$ of imipramine and 0.7–35 μg $ml^{-1}$ of desipramine.

Some results are shown in Tables 1 and 2. The spectrophotometric method was characterised by the molar coefficients $2.24 \times 10^4$ l mol $^{-1}$ $cm^{-1}$ for imipramine and $2.34 \times 10^4$ l $mol^{-1}$ $cm^{-1}$ for desipramine with correlation coefficients of 0.9998 and 0.9997 for imipramine and desipramine, respectively. The regression equations for each drug are, intercept 0.0247 and 0.0413, slope 0.0711 and 0.0769 for IMI and DES, respectively.

The lower limit of the method was found to be 0.07 and 0.11 μg $ml^{-1}$ for imipramine and desipramine, respectively.

The reproducibility of the measurements expressed as relative standard deviation (RSD) varied to 0.5% for the concentrations of the drugs at the examined levels.

*3.2.1. Effect of interferences*

In order to evaluate the selectivity of the developed method for the analysis of pharmaceutical

preparations, the effect of the presence of several species with can occur in the real samples with imipramine and desipramine were investigated.

A level of interferent was considered to be acceptable if the error was not longer than 2%. No interferences were observed in the determination of the studied drugs in the presence of the common expicients of the tablets (e.g. talc, sodium saccharin, starch, lactose, glucose) and injection (e.g. sodium chloride, sodium pirosulfite, sodium sulfate (**IV**), glucose, ascorbic acid).

### 3.3. Application to pharmaceutical preparations

In order to confirm the applicability of the proposed method, dibenzoazepines were determined in pharmaceutical preparations — injections and tablets of imipramine (Imipramin, Polfa, Poland) and tablets of desipramine (Pertofran, Geigy, Great Britain).

The results obtained for tablets and injections analysis were compared with those given by official methods [16,17].

The proposed method was successfully applied to the determination of IMI in injections or tablets and desipramine in tablets. The results of the spectrophotometric assay injections and tablets compared favourably with those obtained using official methods; for a declared content of 10 or 25 mg for tablets and 25 mg/2 ml for injections, the proposed method gave a mean result ± RSD of 10.14 mg ± 0.38% or 24.86 mg ±

Table 1
Spectrophotometric determination of IMI, $n = 6$[a]

| Concentration of IMI (μg ml$^{-1}$) | $\bar{A}$ | Standard deviation $S$ | Standard deviation of the mean $\bar{S}$ | Confidence interval $\mu = \bar{A} \pm t\bar{S}$ |
|---|---|---|---|---|
| 0.32 | 0.036 | 0.0016 | 0.0009 | 0.036 ± 0.0023 |
| 0.63 | 0.059 | 0.0024 | 0.0014 | 0.059 ± 0.0036 |
| 0.95 | 0.084 | 0.0020 | 0.0012 | 0.084 ± 0.0031 |
| 1.58 | 0.136 | 0.0041 | 0.0024 | 0.136 ± 0.0062 |
| 3.17 | 0.243 | 0.0102 | 0.0059 | 0.243 ± 0.0152 |
| 9.51 | 0.735 | 0.0135 | 0.0078 | 0.735 ± 0.0201 |
| 15.85 | 1.164 | 0.0137 | 0.0079 | 1.164 ± 0.0203 |
| 22.18 | 1.617 | 0.0159 | 0.0092 | 1.617 ± 0.0237 |
| 31.69 | 2.256 | 0.0218 | 0.0126 | 2.256 ± 0.0324 |
| 39.61 | 2.839 | 0.0194 | 0.0112 | 2.839 ± 0.0288 |

[a] Tabulated student's $t$-test is 2.571 for a confidence interval of 95% with $(n-1)$ degrees of freedom.

Table 2
Spectrophotometric determination of DES, $n = 6$[a]

| Concentration of DES (μg ml$^{-1}$) | $\bar{A}$ | Standard deviation $S$ | Standard deviation of the mean $\bar{S}$ | Confidence interval $\mu = \bar{A} \pm t\bar{S}$ |
|---|---|---|---|---|
| 0.61 | 0.069 | 0.0035 | 0.0020 | 0.069 ± 0.0051 |
| 1.51 | 0.148 | 0.0031 | 0.0018 | 0.148 ± 0.0046 |
| 3.03 | 0.301 | 0.0046 | 0.0027 | 0.301 ± 0.0069 |
| 4.54 | 0.379 | 0.0172 | 0.0099 | 0.379 ± 0.0255 |
| 6.06 | 0.501 | 0.0075 | 0.0043 | 0.501 ± 0.0111 |
| 15.14 | 1.210 | 0.0114 | 0.0066 | 1.210 ± 0.0170 |
| 21.20 | 1.705 | 0.0176 | 0.0102 | 1.705 ± 0.0262 |
| 30.28 | 2.366 | 0.0175 | 0.0101 | 2.366 ± 0.0260 |
| 37.85 | 2.938 | 0.0243 | 0.0140 | 2.938 ± 0.0360 |

[a] Theoretical value of student's $t$-test is 2.571 ($\alpha = 95\%$).

0.28% and 25.08 mg ± 0.24%, and official method 10.12 mg ± 0.48%, 25.16 mg ± 0.36%, 24.96 mg ± 0.32%, respectively, the relative error of the spectrophotometric method with respect to official methods is less than 0.5%.

Satisfactory recovery values ranging from 99.84 to 100.48% in Imipramin and 99.78 to 100.72% in Pertofran were obtained for IMI and DES investigated from pharmaceutical preparations, respectively.

The proposed method is superior to other conventional methods in that it is fast and simple. The described method of the determination of IMI and DES may be a complement to the applied methods. Reagent consumption is minimum, instrumentation is available in any analytical laboratory without needing expensive laboratory equipments, precision and reproducibility of the adopted spectrophotometric systems are good, and the values of RSD are low.

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