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SPECTROPHOTOMETRIC DETERMINATION OF SOME PHARMACEUTICAL COMPOUNDS USING 2,2-DIPHENYL-1-PICRYLHYDRAZYL

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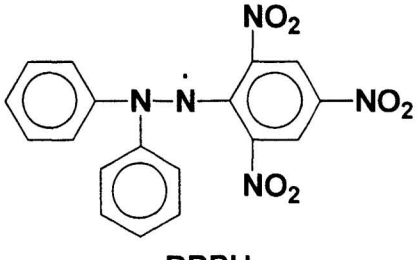
ABSTRACT

A simple, rapid and sensitive spectrophotometric procedure for the assay of some drugs has been developed. The method is based on the reaction of the studied drugs with 2,2-diphenyl-1-picrylhydrazyl (DPPH). The latter is employed to abstract a hydrogen atom from the drugs thereby promoting a process of radical coupling. This results in a reduction of the violet color of DPPH with the formation of the yellow colored 2,2-diphenyl-1-picrylhydrazine ($DPPH_2$). The decrease in the intensity of the violet color is used to measure the concentration of the drugs. All measurements are made at λ= 520 nm on methanolic solutions of the reagent and drugs. Beer's law is obeyed in the ranges of 5-30 μg/ml (for aceclofenac, diclofenac sodium and thiaprofenic acid), 2-15 μg/ml (for tenoxicam, furosemide and lansoprazole) and 2-12 μg/ml (for benoxinate hydrochloride and ritodrine). The validity of the method was tested by carrying out standard addition procedure analyzing the studied drugs in pure form as well as in their pharmaceutical preparations without interference from common additives. Results of the proposed methods are in good agreement with those of the official or reported methods.

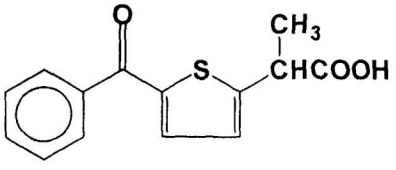
INTRODUCTION



DPPH

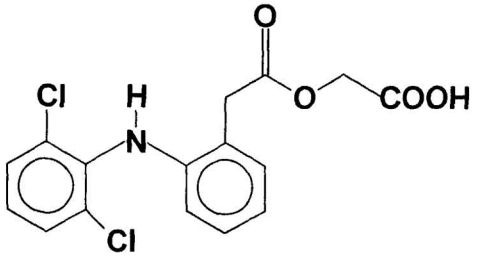
2,2- Diphenyl-1-Picrylhydrazyl (DPPH) is an intense, violet-colored, stable, free radical which reacts as a chromogenic

reagent[1,2] by abstracting a hydrogen atom from the analyt to form yellow colored N,N-diphenylpicrylhydrazine ($DPPH_2$). This decrease in the intensity of the violet color is used as a measure of the quantity of the tested drugs. The radical DPPH was chosen for the present work because it does not dimerize[3] and the problem of cage effect does not arise. In addition, it is deeply colored and its concentration at any time can be estimated by its absorption in the visible range[4].



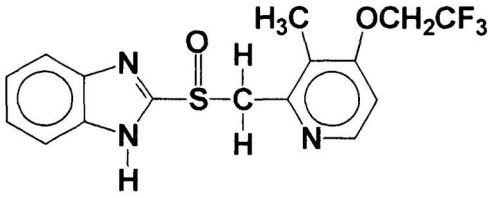
Thiaprofenic acid

Thiaprofenic acid is an anti-inflammatory, antipyretic drug with analgesic properties. Its chemical structure is 2-(5-benzoyl-2-thienyl)propionic acid. Several techniques were used for the determination of this drug such as spectrophotometric[5], polarographic[6], nuclear magnetic resonance[7] , high pressure liquid chromatographic[8,9] and atomic absorption spectrophotometric methods[10].



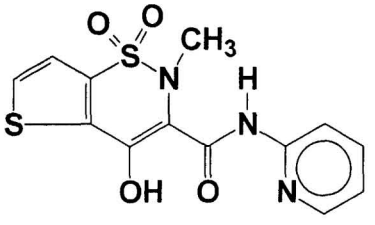
Aceclofenac

Aceclofenac has potent analgesic, anti-inflammatory and antipyretic effects. Its chemical structure is 2-[{2-(2-((2,6- dichloro-phenyl)amino)1-phenyl)acetyl}oxy]acetic acid. Determination of aceclofenac using adsorptive stripping voltammetric techniques on conventional and surfactant chemically modified carbon paste electrodes have been reported[11].



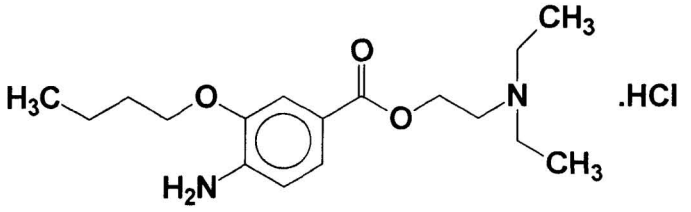
Lansoprazole

Lansoprazole is a substituted benzimidazole, it is one of the first drugs of a new class of orally active anti-ulcer agents. Its chemical structure is 2-[[[3-methyl-4-(2,2,2-trifluoroethoxy-2-pyridinyl]methyl]sulfinyl]-1H-benzimidazole. Several chromatographic[12-14] and spectrophotometric[15,16] techniques were used for its analysis.



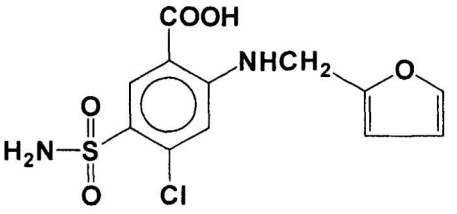
Tenoxicam

Tenoxicam is a potent non-steroidal, anti-inflammatory, anti-rheumatic and analgesic drug. Its chemical structure is 4-hydroxy-2-methyl-N-(2-pyridyl)-2H-thieno[2,3-e]-1,2-thiazine-3-carboxamide1,1-dioxide. Polarographic[17], spectrophotometric[18,19] and chromat-ographic[20,21] methods were recommended for the determination of tenoxicam.



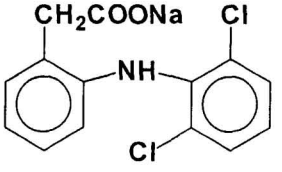
Benoxinate hydrochloride

Benoxinate hydrochloride is indicated as corneal anaesthesia of short duration. Its chemical structure is 2-(diethyl-amino)ethyl4-amino-3-n-butoxybenzoate hydrochloride. Spectrophotometric[22] and pharmacological[23] methods were recommended for the determination of benoxinate hydrochloride.



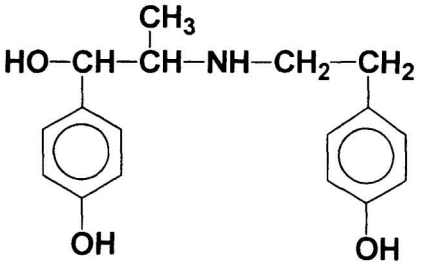
Furosemide

Furosemide is the most potent diuretic available. It is therapeutically used in cases of acute pulmonary edema, acute renal failure and hypercalecemia. Its chemical structure is 4-chloro-N-furfuryl-5-sulfamoyl anthranilic acid[24]. Pharmacological[25], chrom-atographic[26-28] and spectrophotometric[29-33] methods have been reported for the assay of furosemide.



Diclofenac sodium

Diclofenac acid is widely used as an anti-inflammatory agent. Its chemical structure is [2-(2,6-dichloroanilino-phenyl]acetic acid. Several techniques have been used for the determination of this drug including chromatographic[34-36] and spectrophotometric methods[33,37,38].



Ritodrine

Ritodrine is [erythro-2-(4-hydroxyphenethylamino)-1-(4- hydr-oxyphenyl)propan-1-ol] is a sympathomimetic drug, used as an uterine relaxant drug.

It inhibits the frequency and intensity of uterine contractions. Different methods, reported for determination of ritodrine, including pharmacological[39], chromatographic[40-43] and spectrophotometric techniques[44] .

In this work, a simple, rapid and sensitive spectrophotometric method is adopted for the determination of aceclofenac, thiaprofenic acid, tenoxicam, lansoprazole, furosemide, ritodrine, diclofenac sodium and benoxinate hydrochloride in the pure forms and in pharmaceutical preparations with 2,2-diphenyl-1-picrylhydrazyl (DPPH).

EXPERIMENTAL

Apparatus

A Shimadzu UV1601, UV-visible spectrophotometer (Tokyo, Japan) and Memert type thermostatically controlled water bath (Germany) were used. All volumetric measurements were made with standard glassware.

Materials

All solvents and reagents were of analytical reagent grade. The pharmaceutical grade pure drugs; aceclofenac was obtained from Bristol-Myers Squibb (Squibb, Egypt), furosemide, lansoprazole and thiaprofenic acid were obtained from Hoechst (Hoechst, Egypt), diclofenac sodium and ritodrine were obtained from Pharco (Pharco, Egypt) and benoxinate hydrochloride and tenoxicam were obtained from Eipico (E.I.P.I.Co, Egypt). All compounds were complying with requirements recommended by official or other reported methods and used as such without further purification. A standard stock solution of 50 mg/100 ml of each studied drug was prepared in methanol.

Reagents and solutions

2,2-Diphenyl-1-picrylhydrazyl (Sigma, St.Louis, Mo, USA). A stock solution of 1.5 mg/ ml was prepared by dissolving 0.15 g of DPPH in methanol and then diluted to 100 ml with the same solvent. Ten milliliter of this solution were diluted to 100 ml to give 0.15 mg/ ml (working DPPH solution). The stock and working

solutions were kept in a refrigerator and protected from light. The solution was found to be stable for at least one week at 4°C. All chemicals and solvents were of analytical grades.

Pharmaceutical preparations

The commercial dosage forms subjected to analysis were Epicotil tablets (labeled to contain 20 mg tenoxicam per tablet), Epicotil suppositories (labeled to contain 20 mg tenoxicam per suppository) and Epicotil vials (labeled to contain 20 mg tenoxicam per vial),E.I.P.I.Co, Egypt; Soral capsules (labeled to contain 20 mg tenoxicam per capsule) and Soral suppositories (labeled to contain 20 mg tenoxicam per suppository), Global Napi Pharmaceuticals (under license of help Ltd-Greece); Benox ophthalmic solution (labeled to contain 4 mg benoxinate hydrochloride per each ml), E.I.P.I.Co, Egypt; Lanzore capsules (labeled to contain 20 mg lansoprazole per capsule), Hoechst Co., Egypt; Bristaflam tablets (labeled to contain 100 mg aceclofenac per tablet), Squibb Co., Egypt; Surgam tablets (labeled to contain 100 mg thiaprofenic acid per tablet), Surgam suppositories (labeled to contain 100 mg tiaprofenic acid per suppository) and Surgam vials (labeled to contain 300 mg thiaprofenic acid per vial), Hoechst Co., Egypt; Lasix tablets and ampoules were obtained from Hoechst Co., Egypt, labeled to contain 40 mg and 20 mg furosemide per tablet and ampoule, respectively. Declophen tablets and ampoules, Pharco Co., Egypt, labeled to contain 25 mg and 75 mg diclofenac sodium per tablet or ampoule respectively, were used. Yutopar tablets and ampoules (Pharco Co., Egypt), labeled to contain 10 mg of ritodrine hydrochloride per tablet and 1 ml of ampoule, respectively, were used.

General procedure

Transfer 1 ml of the investigated drug to a 10 ml volumetric flask, add 2 ml of DPPH solution. Mix well for lansoprazole and diclofenac sodium at 25°C, heat in water bath at 60°C for 15 minutes for aceclofenac, thiaprofenic acid, furosemide and benoxinate hydrochloride and at 40°C for 10 minutes for tenoxicam and ritodrine. Cool and complete to the mark with methanol. Measure the absorbances of the sample and a reagent blank against methanol at λ=520 nm. Calculate ΔA, i.e. the difference between the absorbance values of the blank and sample which corresponds to the drug concentration.

Assay of pharmaceutical preparations:

Assay of tablets

Twenty tablets were accurately weighed and finely powdered. An amount equivalent to about 50 mg of each drug was weighed accurately. The powder was transferred to 100 ml volumetric flask, extracted successively three times with 10 ml of methanol and completed to the mark with the same solvent. The extract is diluted to obtain 50 μg/ml before carrying out the general procedure.

Assay of capsules

The contents of twenty capsules were weighed and finely powdered. An amount equivalent to 50 mg of each drug was weighed accurately and transferred into a 100 ml volumetric flask. Thirty milliliter of methanol are added to the flask , which was shaken for 10 min. The methanolic supernatant solution is filtered into a 100 ml volumetric flask. The extraction is repeated twice and the content of the volumetric flask is diluted to volume with the same solvent. An accurately measured volume of this solution is pipetted into 10 ml volumetric flasks then completed as under general procedure.

Assay of suppositories:

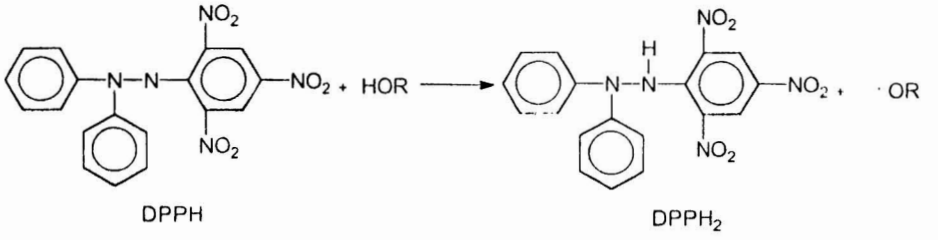
Five suppositories for each drug were accurately weighed and cut into small pieces. The suppository mass was transferred into a porcelain dish and melted on a boiling water bath to complete homogenity. An amount of the suppository mass equivalent to 50 mg of each drug was mixed with 25 ml methanol and shaken for 5 min. The solution was filtered into a 100 ml volumetric flask and completed to volume with methanol and completed as under general procedure.

Assay of ampoules and eye drops

Aliquot of ampoules or eye drops equivalent to 50 mg of each drug was transferred into a 100 ml volumetric flask and completed to volume with methanol and completed as under general procedure.

RESULTS AND DISCUSSION

The ultraviolet-visible spectra of the assay solution of one of the tested drugs, DPPH and $DPPH_2$ are shown in Figure 1. The reaction is assumed to proceed via abstraction of hydrogen atoms from the drugs by DPPH[45]. This is accompanied by the change of violet color of DPPH to give the yellow colored $DPPH_2$ and the corresponding free radical of the drug (Scheme 1).



Scheme 1

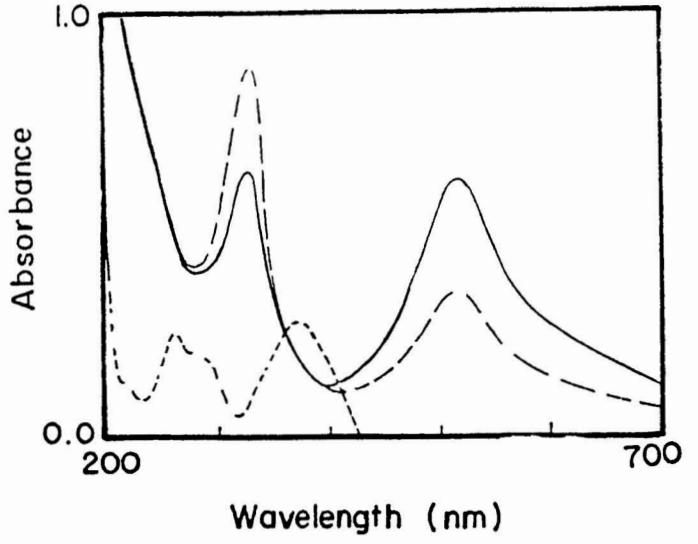


Fig.1: Absorption spectra of(—) DPPH, 30 μg/ml, reaction mixture of DPPH - tenoxicam (15μg/ml)(----) and tenoxicam 15μg/ml (·····)

Optimization of variables:

Reagent concentration

DPPH is added in excess to react with drugs to be analyzed. By measuring the excess reagent, the consumed DPPH would correspond to the amount of the drug.

The concentration of the reagent that gives the highest absorption value within the participle sensitivity range of absorbance was found to be 0.15 mg/ml. Two milliliters of this solution per 10 ml of the reaction mixture was used.

Reaction time and temperature

The reaction time was determined for the interaction of each studied drug with DPPH solution by following the color reduction at ambient temperature (25 ± 2 °C), 30, 40, 50 and 60 °C.

The optimum temperature and reaction time were recorded in table 1. The ΔA reached a constant level, at once and remained constant for at least 180 minutes.

Molar ratio of the reaction

The stoichiometry of the reaction was assessed by the mole-ratio method[46] and the results have been appeared in Table 1. Such results obtained have been cover the number of abstractable hydrogen present in the drug molecule.

Table 1. Assay parameters of the studied drugs.

Drug	Heating time (min.)	Temp. (°C)	Molar ratio of Drug-DPPH*
Ritodrine	10	40	1:2
Furosemide	15	60	1:1
Diclofenac sodium	-	25	1:1
Aceclofenac	15	60	1:1
Lansoprazole	-	25	1:1
Tenoxicam	10	40	1:2
Thiaprofenic acid	15	60	1:1
Benoxinate hydrochloride	15	60	1:1

* Equimolar concentration=$4x10^{-4}$M

Influence of solvents

The effect of dilution of the reaction product by different solvents namely, methanol, ethanol, n-propanol, isopropanol, n-butanol, acetone was studied. The results indicated that all solvents had no effect on the position of maximum absorption while the reactivity (ΔA value) was affected. Methanol was found to be the most suitable solvent.

Quantification

Under the optimum parameters, Beer's law was obeyed for all studied drugs in the range shown in Table 2. The regression analysis of ΔA value versus concentration were done for all the studied drugs according to the linear regression equation summarized in Table 2.

Table 2. Spectral characteristics of the studied drugs by the proposed method.

Drug	Drug Linear range (Ug/ml)	Regression analysis		
		B	K	R
Ritodrine	2-12	0.0012	0.1545	0.9999
Furosemide	2-15	-0.0215	0.2547	0.9997
Diclofenac sodium	5-30	-0.1254	0.1589	0.9994
Aceclofenac	5-30	0.0100	0.1478	0.9995
Lansoprazole	2-15	0.0033	0.1235	0.9996
Tenoxicam	2-15	0.0147	0.2154	0.9999
Thiaprofenic acid	5-30	0.0121	0.1963	0.9996
Benoxinate HCl	2-12	-0.0951	0.1157	0.9991

B: Intercept, K: Slope and R: Correlation coefficient.

Effect of pH

The effect of pH of the added acetate buffer on color reduction was studied for the investigated drugs. The results revealed that the reaction was independent on pH. Because of this independence of the reaction on pH, further investigations were not carried out to establish whether the constituents or pH range

of the buffer solutions have any effect on the interaction of DPPH with the investigated drugs.

Interference

Before proceeding with the analysis of the studied drugs in dosage forms, interference abilities from added common excipients (such as lactose, sucrose, starch, magnesium stearate and gum acacia) were carried out to explore their effect. Samples were prepared by mixing known amounts of the investigated drugs with various amounts of the common excipients.

The good percentage recoveries of the investigated drugs obtained from those synthetic mixtures show that no interference from these additives takes place with the proposed method. Moreover, accuracy of the suggested procedure was further checked by applying standard addition technique.

The results obtained (Table 3) reveal a high degree of accuracy.

Table 3. Assay of the studied drugs in presence of common excipents by the proposed method applying the standard addition technique.

	Recovery % ±S.D.			
Drug	**Sucrose 20mg***	**Glucose 10mg ***	**Lactose 10mg***	**Starch 25mg***
Ritodrine	99.1±0.25	98.4±0.19	99.3±0.75	100.1±0.31
Furosemide	98.9±0.98	99.2±0.69	98.9±0.87	99.6±0.39
Diclofenac	98.9±0.95	99.8±.0.65	99.9±1.20	100.0±0.32
Aceclofenac	99.8±0.45	99.8±0.26	98.4±0.89	99.9±0.98
Lansoprazole	100.1±0.88	98.7±0.69	99.5±0.78	98.7±1.11
Tenoxicam	99.4±0.33	98.7±0.78	100.1±0.75	98.1±0.22
Thiaprofenic	98.9±0.29	99.4±0.37	100.3±1.33	98.1±0.87
Benoxinate	99.7±0.91	100.6±0.81	98.8±0.39	99.7±0.87

* The amount of excipients added per 50 mg of drug

Assay of pharmaceutical dosage forms

It is evident from the results obtained previously that the proposed method gave satisfactory results with the drugs in bulk (Table 4). Thus different pharmaceutical formulations containing the investigated drugs were analyzed for the content of each drug by the proposed procedure as well as the official or reported methods. Data recorded in Table 4 showed no interference by excipients and additives. The results of the analysis recorded in Table 5 indicate the suitability of the method to the assay of the drugs in different pharmaceutical dosage forms. The calculated values of F and t (at 95% confidence level) did not exceed the tabulated (theoretical) ones. This means that there is no significant difference between the proposed and official or reported methods with respect to precision and accuracy.

Table 4. Recovery studies at different drug concentrations for all the studied drugs.

	Ritodrine	Furosemide	Benoxinate HCl	Diclofenac sodium
$\bar{X}$	100.45	99.98	100.02	100.21
±S.D.	0.35	0.81	0.71	0.61
RSD	0.35	0.81	0.71	0.60
N	6	6	6	6
V	0.12	0.66	0.50	0.37
	Aceclofenac	**Lansoprazole**	**Tenoxicam**	**Thiaprofenic acid**
$\bar{X}$	99.89	99.95	100.21	100.56
±S.D.	0.94	0.97	0.61	0.74
RSD	0.94	0.97	0.60	0.73
N	6	6	6	6
V	0.88	0.94	0.37	0.55

$\bar{X}$: Mean, S.D.: Standard Deviation, RSD: Relative Standard Deviation, N: Number of experiments, V: Variance

Table 5. Determination of the drugs in their pharmaceutical preparation by the proposed, reported and official methods.

Pharmaceutical Preparations	Recovery % ±S.D.			
	DPPH method	Official or Reported method	t (3.58)	F (4.28)
Epicotil tablets (contains 20mg tenoxicam/tablet)	99.8±0.25	99.9±0.34[47]	0.69	2.00
Epicotil suppositories (contains 20mg tenoxicam/suppository)	100.1±0.56	99.9±0.34[47]	0.62	2.58
Epicotil vials (contains 20mg tenoxicam/vial)	100.0 ±0.44	99.9±0.34[47]	0.39	1.58
Soral capsules (contains 20mg tenoxicam/capsule)	99.4±0.57	99.9±0.34[47]	1.51	2.70
Soral suppositories (contains 20mg tenoxicam/suppository)	99.8±0.31	99.9±0.34[47]	0.56	1.20
Benox solution (Contains 4mg benoxinate HCl/ml)	100.2±0.94	99.4±1.00[48]	0.37	1.14
Lanzore capsules (Contains 20mg lansoprazole/capsule)	98.7±0.74	99.1±0.88[47]	0.94	1.40
Bristaflam tablets (Contains100mg aceclofenac/tablet)	100.2±0.17	100.1±0.21[47]	1.02	1.33
Surgam tables (Contains 100mg thiaprofenic acid/tablet)	99.1±0.75	99.3±.0.80[37]	0.62	1.14
Surgam suppositories (Contains 100mg thiaprofenic acid/suppository)	99.8±0.77	99.3±.0.80[37]	1.12	1.08

Continue Table 5. Determination of the drugs in their pharmaceutical preparations by the proposed, reported and official methods.

Pharmaceutical Preparations	Recovery % ±S.D.			
	DPPH method	Official or Reported method	t (3.58)	F (4.28)
Surgam vials (Contains 300mg thiaprofenic acid/vial)	99.6±0.89	99.3±.0.80[37]	0.58	1.23
Lasix tablets (contains 40mg furosemide/tablet)	100.5±0.99	100.2±0.89[24]	0.52	1.24
Lasix ampoules (Contains 20mg furosemide/ampoule)	99.7±0.78	100.2±0.89[24]	1.11	1.30
Declophen tablets (Contains 25mg declofenac acid/tablet)	99.1±29	99.3±0.30[37]	1.19	2.25
Declophen ampoules (Contains 75mg declofenac acid/ampoule)	99.0±0.23	99.3±0.30[37]	2.25	1.80
Yutopar tablets (Contains 10mg ritodrin/tablet)	100.4±0.93	100.5±1.00[37]	0.19	1.16
Yutopar ampoules (Contains 10mg ritodrine/ampoule)	100.3±0.89	100.5±1.00[37]	0.39	1.27

In conclusion, the DPPH method is simple, sensitive, accurate and precise. It also has the advantage of being applicable to pharmaceutical preparations. In comparison to the official or reported methods, the DPPH method is rapid, specific and more sensitive. In addition, the DPPH reagent is stable, doesn't dimerize and is ready for use immediately and its reaction with the selected drugs is rapid and the reaction products are stable for more than two hours.

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