First Order Derivative Spectrophotometric Determination of Orphenadrine Citrate in Pure and Pharmaceutical Preparation

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Summary: An improved first order derivative spectrophotometric method for the determination of orphenadrine citrate in pure and pharmaceutical preparation was developed. Orphenadrine citrate reacts with 1-amino naphthalene and sodium nitrite, after heating for 180s at 70°C to give a yellow colour having maximum absorbance at 476.3 nm. The reaction is selective for orphenadrine citrate with 4.0 µg/mL as visual limit of quantitation. The color reaction obeys Beer's Law from 4.0 µg/mL-230 µg/mL of orphenadrine citrate and the relative standard deviation is 0.57%. The procedure does not require any separation step. Mean recoveries were found to be greater than 99.5% in the method for orphenadrine citrate in its synthetic mixtures. The quantitative assessment of tolerable amount of other drugs was also studied. Doubly distilled water or analytical grade ethyl alcohol was used as a solvent throughout the study.

Introduction

Orphenadrine citrate [1] is N, N-dimethyl-2-[o-methyl-α-phenyl benzyl) oxy] ethylamine citrate (Fig .1). Orphenadrine, a methyl analogue of antihistamine has weak antihistaminic and mild anticholinergic activities. Early pharmacological reports noted that the drug reduces voluntary muscle spasms by a central effect, and possible utility in parkinson's disease was suggested. Indications for citrate are given as adjunct for the relief of discomfort associated with acute painful muscloskeletal conditions by a mode of action not early identified but they may be related to analgesic property of the compound [2]. There are few analytical studies reported in the literature for the determination of orphenadrine citrate in pharmaceutical samples and biological fluids by spectrophotometer [3], GC [4-5], GC/MS [6], GLC [7], HPLC [8]. Orphenadrine is able to prevent the neurotoxicity mediated by activation at glutamate ionotropic receptors [9]. The efficacy and safety of a diclofenac/orphenadrine were assessed by measuring the subjective pain intensity on a visual analogue scale and on ordinal rating scale before and after infusion [10]. Expression and inhibition of cytochrome P450 (CYP) isozymes capable of forming an orphenadrine metabolite complex were studied in microsomes of untreated and inducer – treated male and female rats [11]. Blink reflex recovery cycle, before and after acute administration of orphenadrine chlorhydrate was tested in Meige syndrome [12]. A double - blind trail of a combination tablet containing orphenadrine and paracetamol, 'Norgesic' was carried out to assess the value of this product compared with paracetamol alone [13]. The effect of intravenous administration of orphenadrine citrate versus placebo on spastic hyper tonic after spinal cord injury was made using the Ashworth spasticity scale [14]. Various studies of acute poisoning and death with orphenadrine have been reported in the literature [15-18]. We are currently engaged in the systematic study of the drug of abuse and it was found that orphenadrine citrate reacts with 1-amino naphthalene and sodium nitrite, to give a yellow colour having maximum absorbance at 476.3nm. The paper describes a validated method for the qualitative determination of orphenadrine citrate in tablets using first order derivative spectrometery.

Results and Discussion

Absorption Spectrum of Coloured Complex

Orphenadrine citrate reacts with 1-amino naphthalene and sodium nitrite when heated at 70°C
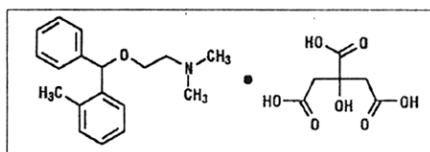

Fig. 1: Structure formula of orphenadrine citrate.
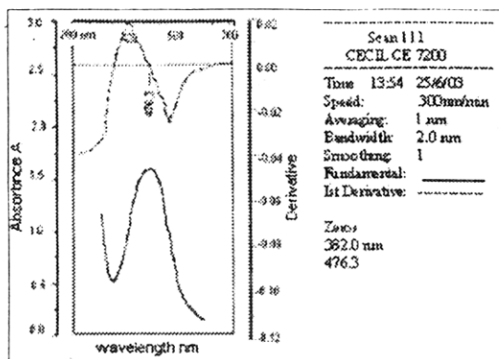
*To whom all correspondence should be addressed.

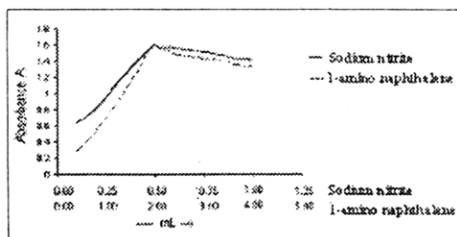Fig. 2: First order derivative spectrum of orphenadrine citrate.

Fig. 3: Effect of colour producing reagents.

Fig. 4: Effect of temperature on the stability of colour.
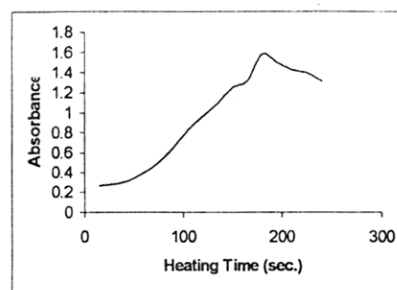
Fig. 5: Effect of heating time on the stability of colour.

for 180s to give a yellow coloured complex, the first order derivative spectrum of which lies under optimum condition at 476.3 nm (Fig.2).

Effect of Colour Producing Reagents

It was found that 1 mg/10mL of (0.05%) 1-amino naphthalene and 5mg/10mL of (1%) sodium nitrite gave maximum colour (Fig. 3). If the concentration of these reagents were changed the colour intensity was diminished, and if either one of the two reagents was absent, then too, the colour did not develop. There is no effect of ethyl alcohol used for dissolving 1-amino naphthalene upon this particular colour reaction.

Effect of Temperature and Heating Time

The effect of temperature is shown in Fig. 4. It was found that with the rise of temperature the colour intensity was increased and was stable at 70°C, however, above this temperature the colour intensity decreased. The colour also developed at room temperature within 15 s but of low intensity. The absorbance of the developed colour remained s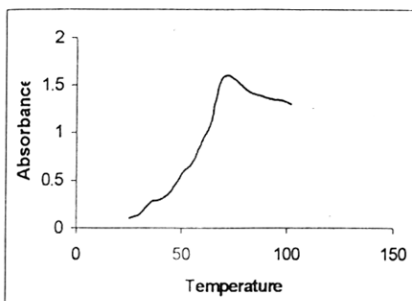table for more than 120 min. A water bath was used to carry out the temperature studies. The effect of the heating time on the colour intensity was shown in the Fig. 5. It was shown that the heating for 180s at 70°C gave the maximum colour intensity. Above and below these times there was a slight decrease in colour intensity. The contents of the test tube were cooled to room temperature prior to dilution to 10mL with ethanol and measurement of absorbance.

Effect of Organic Solvents

Different organic solvents such as benzene, chloroform, acetone and methanol were tested for colour extraction and for stability, but none was as effective as ethanol. The colour was stable for more than 120 min. Without the addition of any solvent. Ethanol was used for dilution up to 10mL of the coloured complex so as to avoid any formation of emulsion.

Sensitivity

The results for the determination of pure orphenadrine citrate are shown in the Tables-1 & 2, which show the sensitivity, validity, and repeatability
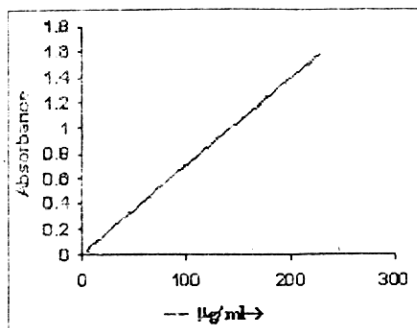
Fig. 6: Calibration curve for orphenadrine citrate.

Table-1:Determination of orphenadrine citrate from pure solution.

Orphenadrine citrate taken	Orphenadrine citrate found	Relative standard deviation
(mg/ml)	(mg/ml)	(%)
0.1	0.090	0.24
0.5	0.502	0.54
1.0	1.040	0.15
1.5	1.509	0.57
2.0	2.015	0.50

Every value is an average of five readings.

Table-2: Comparative regression line and correlation parameters for the determination of orphenadrine citrate by ($^1D_{476.3nm}$) first derivative method.

Parameters	Values
Analytical method	$^1D_{476.3nm}$
Beer's law limit (µg/mL)	4.0-230
Molar absorptivity (mol $^{-1}$cm^{-1})	3.18×10^3
Regression equation (Y)*	$Y = 5.50 \times 10^{-3} + 0.67C$
Correlation coefficient (r)	0.960
RSD** (%)	0.57
Confidence Limit at 95% confidence level	5.92±0.05

*Y = a + bC   Where C is the concentration of analyte (1mg/10mL), 'a' is intercept and 'b' is the value of slope.
**Calculated from five independent determinations.

Table-3:Quantitative assessment of tolerable amount of other drugs.

Drugs	Maximum mount not interfering (%)
Temazepam	40
Phenol barbitone	30
Brufen	80
Erythrocine	100
Hydroxyzine	120
Metroteine HCl	80
Atenolol	25

The value is percentage of drug with respect to 1mg/ml of orphenadrine citrate that causes ±0.01 changes in absorbance.

of the method. It is also reasonably precise and accurate as the amount taken from identical samples is known and the amount found by the above procedure does not exceed the relative standard deviation 0.57% which is replicate of five determinations (cf. Table-1). The optimization has been done at lower analyte concentration. The calibration graph is linear in the range of 4.0 µg-230 µg/mL. The apparent molar absorptivity calculated was 3.18×10^3 mol^{-1} cm^{-1} and the regression equation calculated by the method of least squares from 14 points, each of which was an average of five determinations.

Interferences

The quantitative assessment of tolerable amount of other organic compounds (w/v) under experimental conditions is given in the Table-3. Various amount of diverse interfering compounds were added to the fixed amount of orphenadrine citrate (1mg/mL) and the procedure for the derivative spectrophotometric determination was followed.

Application

The proposed method is successfully applied for the quality control of pure orphenadrine citrate and in pharmaceutical dosage form as shown in the Table 4.

Experimental

Apparatus

Cecil CE 7200 UV-Visible Derivative Spectrophotometer with data processing capacity was used. Visible spectra of reference and test solutions were recorded in 10mm optical glass cells at a fixed slit width of 2nm. Midac USA. FTIR Spectrophoto-meter and graduated pipettes were also employed.

Reagents and solutions

The active substance orphenadrine citrate was tested for its purity by checking its m.p (136°C) and IR-Spectrum (which is concordant with the reference structure of orphenadrine citrate) and no impurity was found.

Orphenadrine citrate (Wilson's Pharma, Islamabad, Pakistan) standard solution (w/v) (1 mg/mL) was prepared in doubly distilled water to give a stock solution, which was diluted further as required.

05% (w/v) 1-amino naphthalene (E.Merk) solution was prepared by dissolving 0.05gm of it in 1.5 mL of absolute alcohol (E.Merk) and diluted up to 100 mL with doubly distilled water.

Table-4:Determination of orphenadrine citrate from pharmaceutical preparation (tablets)

Trade name	Manufacturer's specification (mg)	Amount found (mg)	RSD (%)	Recovery studies (%)
Wilgesic (Wilison's Pharma, Pak.)	35	34.98	0.15	99.5
Norgesic (Searle Pak.Ltd.)	35	35.01	0.009	100.04
Nuberol Forte (Searle Pak.Ltd.)	50	49.85	0.34	99.7

Every reading is an average of five independent measurements with five different samples.

1% (w/v) sodium nitrite (BDH) solution was prepared in distilled water.

All the chemicals used were of analytical grade.

General Procedure

To an aliquot (1mL) of orphenadrine citrate containing 4.0μg-230μg/mL was added 2.0mL of 0.05% 1-amino naphthalene and 0.5 ml of 1% $NaNO_2$ and the contents were heated for 180 s in water bath at 70°C, cooled and the volume was made up to 10mL with ethanol. The resulting absorbance of the yellow colour was measured at 476.3 nm, employing all the reagents except orphenadrine citrate as blank. The experiment was repeated with different concentrations of drug and the calibration curve was prepared (Fig.6)

Method for Studying Interferences

To an aliquot (1 mL) containing 1 mg/mL of orphenadrine citrate, different amounts of various interfering compounds (1 mg/mL) (w/v) were added as long as the solution showed ± 0.01 absorbance as the pure orphenadrine citrate solution without the addition of the interfering organic compound under experimental conditions as described in the general procedure. The value was calculated as the percentage of the compound with respect to the amount of orphenadrine citrate.

Method for Determination of Orphenadrine Citrate In Pharmaceutical Dosage

Tablets

Four tablets each containing 50mg of orphenadrine citrate were powered, weighed, and then dissolved in (2:3) distilled water and filtered. The filtrate was diluted to get a 1 mg/mL solution of orphenadrine citrate. An aliquot containing 4.0 μg-230 μg/mL was taken, the procedure was followed as described above and the absorbance measured at 476.3 nm. The quantity per tablet was calculated from the standard calibration graph.

Conclusions

The derivative spectrophotometric method for the determination of orphenadrine citrate is simple, reliable, specific, sensitive and less time consuming. This method can be successfully applied for the trace determination of orphenadrine citrate in pure and pharmaceutical preparations.

The colour reaction has 4.0 μg/mL as visual limit of identification. Thus the present method is reproducible, accurate and precise.

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