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COLORIMETRIC DETERMINATION OF THYMOL, OXYPHENBUTAZONE AND MORPHINE HYDROCHLORIDE

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A simple colorimetric method has been developed for the determination of thymol, oxyphenbutazone and morphine hydrochloride either in pure forms or in pharmaceutical preparations. The method is based on the formation of an azo dye from the condensation of diazotized 5-chloro-2,4-dinitroaniline with the titled drugs which contain a phenolic hydroxyl group. The absorption spectra for the coupling coloured products for thymol, oxyphenbutazone and morphine hydrochloride exhibit absorption maxima at 592 nm (ε=46,153), 450 nm (ε=20,689) and 480 nm (ε 11,764) respectively . For each of the three studied drugs a typical calibration curve was obtained from linear regression analysis of absorbance at various drug concentrations. The developed colour for each drug was stable for at least 30 minutes. Optimum conditions for the colour formation have been determined. The application of this procedure to pharmaceutical preparations is given with good recoveries (99.69-100.50%).

Thymol (antibacterial, preservative and antiseptic), oxyphenbutazone (analgesic and anti-inflammatory) and morphine (narcotic analgesic) are widely used in pharmaceutical practice.

The official compendia describe a volumetric titration for thymol , a titration with standard sodium hydroxide using bromothymol blue as indicator[1] or the end point was determined potentiometrically[2] for oxyphenbutazone, and nonaqueous titration for morphine[1,2]. Among the methods described for the assay of thymol are volumetric[3], colorimetric [4-6] using various reagents and polarographic methods[7].

Oxyphenbutazone was determined by high speed liquid chromatography[8,9], gas liquid chromatography[10], spectrophotometry[11] and mass spectral analysis[12]. The proposed methods for analysis of morphine include a number of gravimetric[13,14] titrimetic[15], spectrophotometric [16-18], polarimetric[19] chromatographic[20], electrophoretic [21], isotope dilution[22], radioimmunoassay-gas liquid chromatography[23], and high pressure liquid chormatography[24,25].

Diazotized 5-chloro-2,4-dinitroaniline has been successfully employed in thes laboratories as a good reagent for the colorimetric determination of vitamin B_6 [26], ethinyl estradiol[27] and certain sympathomimetic drugs[28]. The suitability of this reagent for the colorimetric determination of phenolic drugs; thymol, oxyphenbutazone and morphine hydrochloride has been investigated. Although the structure of these compounds differ considerably, each has a common group upon which the proposed method for determination is based.

As a result of these studies, a rapid, simple and sensitive colorimetric method for the determination of the titled drugs in pure and dosage forms has been developed.

METHODS

1- Equipment:

Spectrophotometer PU2DL (Zeiss, Oberkochen, West Germany)

2- Materials:

All chemicals and reagents used were analytical grade. 5-Chloro-2,4-dinitroaniline was prepared by a reported procedure[29]. Several crystallizations from ethanol yielded an analytical sample m.p. 174°. Thymol, oxyphenbuatazone (Tandril(R)) and morphine hydrochloride were used as working standards without further treatment. Commercial preparations including tablets and ampoules containing oxyphenbutazone and morphine HCl were purchased from the market. Two synthetic mixtures containing thymol were prepared in our laboratory.

3- Diazotized 5-chloro-2,4-dinitroaniline:

Ten mg of 5-chloro-2,4-dinitroaniline were accurately weighed into 10-ml volumetric flask and dissolved in 2 ml of sulphuric acid. After cooling in ice bath, 0.5 ml 2% sodium nitrite solution in distilled water was added. This was allowed to stand 2 minutes and then 0.2 ml 10% w/v sulphamic acid in distilled water was added and completed to volume with distilled water. The contents were mixed and kept in ice bath. This reagent solution should not be used after 5 hours.

4- Standard Solutions:

Fifty mg of (morphine HCl or oxyphenbutazone or thymol) were accurately weighed in 50 ml volumetric flask, dissolved and completed to volume with distilled water. This was the stock solution from which different dilutions were made.

5- Sample Solutions:

For tablets: Twenty tablets were accurately weighed and powdered. An accurately weighed amount of the powder equivalent to 50 mg oxyphenbutazone was transfered to 50-ml volumetric flask, dissolved and completed to volume with distilled water. The contents were either filtered and the first portion was discarded or centrifuged and the supernatant liquid was the stock assay solution.

For liquid preparations: Accurately measured volume of morphine or synthetic mixtures of thymol, equivalent to specific weight for each drug, was quantitatively diluted to obtain the appropriate concentration.

6- Color development:

Into 10-ml volumetric flask, 0.2 ml of the diazotized reagent was pipeted followed by 1.0 ml of aqueous solution of the phenolic drug (thymol 20 ug/ml, oxyphenbutazone 100 ug/ml or morphine HCl 100 ug/ml) and 2.0 ml of N/1 NaOH solution, The contents of the flask were mixed well and the volume was made up to the mark with distilled water. The final solution was measured at the specific λ max for each drug against a blank treated concurrently.

RESULTS AND DISCUSSION

The absorption spectra of the final coupled products of thymol, oxyphenbutazone and morphine HCl with diazotized 5-chloro-2,4-dinitroaniline are shown in Fig. 1.

It is apparent that while both oxyphenbutazone and morphine HCl exhibit absorption maxima at 450 and 480 nm, the thymol absorption maximum appears considerably higher at λ max 592 nm.

The very greater color intensity of the coupled coloured product of thymol can be attributed to the fact that thymol has the most favorable coupling position which is para and ortho to the hydroxyl group. Only coupling ortho to the hydroxyl group is possible for oxyphenbutazone and morphine HCl (Fig. 2). Again, oxyphenbutazone gave higher absorption than morphine because there are two free coupling sites while for morphine there is one site only.

An initial investigation have been made to prepare diazotized 5-chloro-2,4-dinitroaniline in various acids; formic, perchloric, sulphuric and 40% trichloroacetic acids. Table 1 indicates that the coupling coloured product resulting from diazonium salt solution prepared in perchloric and sulphuric acids gave equal absorbances. Sulphuric acid was preferred for its availability. The removal of excess sodium nitrite is essential since it interfered with the colour product and was done by the addition of 10% sulphamic acid solution.

The intensity of absorption was significantly affected by the choice of diluent and the pH of the solution. The colour

intensity of the final solution was increased 20-30% when the drug (thymol, oxyphenbutazone or morphine HCl) to be determined was dissolved in water rather than N/1 NaOH solution, methanol or ethanol. Furthermore, it was found that the intensity of absorbance was significantly greater when using N/1 NaOH solution as diluent (Table 2). It was preferred to use 2 ml N/1 NaOH solution and then complete to 10 ml with distilled water. The volume of diazonium salt solution played an important role on the intensity of the developed colour in the final measured solution. It was found that, 0.2 ml of diazonium salt solution gave the highest absorption intensity for the studied drugs (Table 3).

The effect of time on the absorption intensity of the coupling coloured product at the specific λ_{max} for each drug is shown in Fig. 3. Colour intensity developed immediately and remained constant for 30 minutes in the case of thymol and morphine HCl and for 50 minutes for oxyphenbutazone.

Under these conditions, the absorption spectra of the resulting coloured coupling product for each of the studied drugs with the diazotized solution showed a specific absorption peak at 592, 450 and 480 nm for thymol, oxyphenbutazone and morphine HCl respectively. Beer's law was obeyed for all the studied drugs with high correlation coefficient (Table 4). The slopes of the calibration curves reflect the sensitivity of the colour attributed to azo dye formation. The highest slope, for thymol, parallels its greatest ability to coupling (ortho and para position sites are free).

The specificity of the method for the determination of the titled drugs in the presence of common excipients used in pharmaceutical preparations; fructose, lactose, starch, acacia and magnesium stearate was investigated and showed no interference

at all.

Substances which are usually associated with thymol in pharmaceutical preparations; methyl salicylate, kaolin, boric acid, glycerine, oil of peppermint, menthol, camphor, sodium benzoate, extract tolu, and extract of senega gave no interference when analysed by the proposed method in the concentration found in pharmaceutical preparations.

Morphine HCl may be assayed in the presence of certain related opium alkaloids, papaverine HCl, codeine sulphate and atropine sulphate, without interference since these alkaloids do not contain phenolic groups.

Several formulations available in market along with some synthetic preparations containing thymol, oxyphenbutazone, and morphine HCl were analyzed by the proposed method. Percentage recoveries of the added drugs (Table 5) indicate that the method can be conveniently applied to the products containing the titled drugs.

Table 1: Effect of Preparation of Diazonium Salt Solution in Various Acids on the Absorption Intensity of the Coupling Coloured product with Morphine Hydrochloride

Acid	*Absorbance* at* λ_{max} *480 nm*
Formic acid	no
40% Trichloroacetic acid	0.095
Perchloric acid	0.160
Sulphuric acid	0.160

* Average of 3 determinations.

Table 2: Effect of Different Diluting Liquids on the Intensity of the Coupling Coloured Products of Thymol, Oxyphenbutazone and Morphine Hydrochloride.

Diluent	*Thymol*[b] λ_{max} *592 nm*	*Absorbance*[a] *of Oxyphenbutazone*[c] λ_{max} *450 nm*	*Morphine HCl*[c] λ_{max} *480 nm*
N/1 NaOH ml			
1.0	0.490	0.510	0.120
1.5	0.582	0.530	0.160
2.0	0.582	0.530	0.160
4.0	0.582	0.530	0.160
6.0	0.582	0.530	0.160
Methanol	0.273	0.310	0.085
Ethanol	0.123	0.100	0.015
Acetate Buffer	0.031	0.060	0.012

a Average of 3 determinations
b Final concentration 2 ug/ml
c Final concentration 10 ug/ml

Table 3: Effect of Using different volumes of Diazotized 5-chloro-2,4-dinitroaniline on the absorption intensity of the coupling coloured products

Diazotized 5-Chloro-2,4-dinitroaniline *ml*	*Thymol*[b] λ_{max} 592 nm	*Absorbance*[a] *of Oxyphenbutazone*[c] λ_{max} 450 nm	*Morphine HCl*[c] λ_{max} 480 nm
0.1	0.582	0.530	0.160
0.2	0.582	0.530	0.160
0.3	0.582	0.530	0.160
0.4	0.552	0.500	0.090
0.6	0.488	0.422	0.060
1.0	0.120	0.400	0.020

a Average of 3 determinations
b Final concentration 2 ug/ml
c Final concentration 10 ug/ml

Table 4: Comparative summary of statistical data for the coupling coloured products of the studied drugs

Drug	λ_{max} *nm*	*Linear calibration range* *ug/ml*	*Apparent molar absorptivity* ε	*Correlation coefficient* *r*	*Slope* *b*	*Intercept* *a*
Thymol	592	0.2 - 2.6	46,153	0.992	0.330	0.030
Oxyphenbutazone	450	1.0 - 14	20,689	0.999	0.052	0.004
Morphine HCl	480	2.5 - 15	11,764	0.989	0.020	0.020

Table 5: Application of the proposed method for analysis of the studied drugs in some pharmaceutical formulations.

Formulation	*Claimed*	*Found**		*Added*	*Recovered**	
	mg	*mg*	*%*	*mg*	*mg*	*%*
Tandril tablet (Ciba)	100/tab.	99.17	99.17	100	100.34	100.34
Morphine ampoule (Misr)	20/Amp.	19.82	99.10	20	20.10	100.50
Thymol Nasal solution tablet mixture**	3.24/100 ml	3.20	98.70	5.0	5.01	100.25
Thymol Oropharyngeal preparation**	10/100 ml	9.95	99.50	10	9.90	99.96

* Average of 3 determinations

Composition of formulations:

Tanderil tablets: each tablet contains 100 mg of oxyphenbutazone.

Morphine ampoule : each 1 ml contains 20 mg morphine sulphate

Thymol nasal solution (B.P.C. 1963): Borax 324 mg., sodium bicarbonate 324 mg , and thymol 3.24 mg ,

Thymol oropharyngeal preparation:

Menthol 0.2%, thymol 0.1%, camphor 0.01%, oil of eucalyptus 0.06%, and extract tolu 0.01%

** Synthetic mixtures.

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تقييم لونى للشايمول ، الاكسيفينبيوتازون ،وهيدروكلوريد المورفين

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