Colorimetric Determination of Terbutaline Sulfate in Pharmaceutical Preparations Using Phenanthro[9,10-*d*]imidazole-2-*N*-chloroimide

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A simple and sensitive colorimetric method for the determination of terbutaline sulfate by using phenanthro[9,10-*d*]imidazole-2-*N*-chloroimide (PI-NCl) has been developed.

Terbutaline slfate reacts with PI-NCl at pH 8 without any oxidant to give an indophenol dye having an absorption maximum at 525 nm. A linear calibration plot for terbutaline sulfate was obtained over the concentration range of 0.7—270 μg/ml. The method was applied to the analysis of terbutaline sulfate in commercial pharmaceutical preparations. The relative standard deviations were 0.36% (injection, $n=5$), 0.44% (tablets, $n=5$), and 0.62% (fine granules, $n=5$).

Keywords colorimetric determination; terbutaline sulfate; β_2-adrenergic agent; phenanthro[9,10-*d*]imidazole-2-*N*-chloroimide; drug assay

The resorcinol-type drug terbutaline sulfate (1-[3,5-dihydroxyphenyl]-2-*tert*-butyl-aminoethanol sulfate) is β_2-adrenergic agent used in the treatment of asthma.[1,2] The official compendia describe a nonaqueous titration method for the determination of terbutaline sulfate.[3,4] To determine the amount of the drug in pharmaceutical preparations, a colorimetric method seems to be more suitable for routine analysis in laboratories, although many highly sensitive methods based on high performance liquid chromatography (HPLC)[5-8] and gas chromatography-mass spectrometry (GC-MS)[9,10] have been developed for the analysis of terbutaline in biological fluids and in the degraded products. Several color reactions for phenols[4,11,12] have so far been applied to the determination of terbutaline sulfate. However, these methods are not very sensitive. Among the color reactions, the USP method for the analysis of the drug in tablets and injection formulations is based on a color reaction with 4-aminoantipyrine and potassium ferricyanide.[4] However, the colored compound formed is not stable, so the measurements have to be carefully timed. Recently, Rao and Sastry[13] and El-Yazbi *et al.*[14] reported a sensitive colorimetric method for the determination of terbutaline sulfate in bulk samples and pharmaceutical preparations using *p*-aminophenol–molecular oxygen and 3-methyl-2-benzothiazolone hydrazone hydrochloride (MBTH) in the presence of ferric chloride, respectively. However, the latter method also gives a strong coloration with aromatic amines and aliphatic aldehydes, except for phenolic compounds.

In the previous paper,[15] we reported the synthesis and reactivities of phenanthro[9,10-*d*]imidazole-2-*N*-chloroimide (PI-NCl) as a new color reagent for phenolic compounds. The reagent reacts with resorcinols, 1-naphthol, and dialkylphenols substituted in the 2- and 5- or 6- positions under alkaline conditions without any oxidant to give indophenol dyes having a high molar absorptivity.

This paper reports a sensitive and simple colorimetric method for the determination of terbutaline sulfate and its pharmaceutical preparations based on the formation of a coupled colored product with the PI-NCl reagent, and the present method is compared with the USP method.

Experimental

Apparatus Absorption spectra and absorbance measurements were taken with a Shimadzu UV-260 UV-visible recording spectrophotometer using 10 mm cells.

Materials Terbutaline sulfate standard was supplied by Fujisawa Pharm. Co., Ltd. PI-NCl was prepared by the reaction of 2-aminophenanthraimidazole and sodium hypochloride according to the method of Tanabe *et al.*[15] All other chemicals and reagents were of analytical grade.

Preparation of Assay Solutions Tablets: Ten tablets were weighed and powdered. A quantity of the powder equivalent to 5 mg of terbutaline sulfate was transferred into a 50 ml volumetric flask using 30 ml of 0.01 N HCl. The mixture was extracted by ultrasonication for 30 min, and then 0.01 N HCl was added to volume. The tablet extract was filtered. The first 10 ml of the filtrate was discarded and then collected. The filtrate was diluted with an equivalent volume of water and then subjected to analysis.

Fine Granules: Fine granules were ground to a fine powder. A quantity of the powder equivalent to 5 mg of terbutaline sulfate was transferred into a 50 ml volumetric flask. The procedure described above for tablets was followed.

Injection: A 25 ml quantity of the injection (0.2 mg/ampoule) equivalent to 5 mg of terbutaline sulfate was transferred into a 100 ml volumetric flask and then diluted with water to volume.

Assay Procedure: A 0.25 ml aliquot of the assay solution, 2.0 ml of 0.13 mg/ml PI-NCl in ethanol and 1.5 ml of 50 mM borate buffer (pH 8.0) were mixed in a 10 ml glass test tube. The mixture was allowed to stand for 40 min at ambient temperature, and the absorbance was measured at 525 nm against a reagent blank.

Results and Discussion

PI-NCl is slightly soluble in organic solvents such as ethanol, acetonitrile, and dioxane, and insoluble in water, but soluble in chloroform. In the course of our studies on the determination of phenols with PI-NCl, we have found that the reagent in ethanol gives the stable and highly intense coloration for resorcinol under strongly alkaline conditions.[15] Under the above conditions, the color intensity reached a maximum within 5 min and was stable for at least 90 min. On the other hand, the maximum color formation under a weakly alkaline condition (pH 8.0) was slow (about 40 min) and the molar absorptivity decreased to about 1/10 with a shift of the absorption to a shorter wavelength of about 100 nm. Based on this finding, the reaction of terbutaline sulfate with PI-NCl was carried out in aqueous-ethanol solutions. Terbutaline sulfate gave a strong coloration, showing an absorption maximum at 525 nm at any pH from 7.5 to 13, though the reaction rate between the drug and the reagent and color intensity were affected by the pH and quantities of PI-NCl and ethanol in the reaction solution.

Figure 1 shows the influence of the reaction time at various pHs on the color development of terbutaline sulfate at fixed concentrations of PI-NCl and ethanol. The reaction

rate between terbutaline sulfate and PI-NCl was very fast at higher pH values (pH > 9.0) and the colored solution faded rapidly. Slow fading was observed at pH values less than 9, but the reaction rate for terbutaline sulfate and PI-NCl was very slow at pH 7.5. Stable color development was obtained over the pH range of 7.7 to 8.5; pH 8 was used as an optimum pH. At pH 8.0, the developed color reached maximum after standing for 20 min, and remained stable for at least 50 min. Contrary to the results obtained with resorcinol, no greater absorbance change for terbutaline was observed from pH 7.7 to pH 13.

Color intensities were measured with various concentrations of PI-NCl, up to a 40-fold molar excess (0.25 mg/ml). As shown in Fig. 2, maximum absorbance was obtained when a 15-fold or greater molar ratio of PI-NCl was used. The appropriate concentration of PI-NCl was found to be 0.13 mg/ml by considering the solubility in ethanol and the blank effect.

PI-NCl was dissolved in ethanol, and a maximum and constant value of the color intensity was obtained when a mixture of 2 volumes of ethanol and 1.5 volumes of buffer (pH 8.0) was used. With increasing volume of buffer in the solution, the color faded gradually. All of the other organic solvents examined (dimethylformamide, 1,4-dioxane, tetrahydrofuran, acetone, and methanol) gave color intensities of less than 50% that of ethanol, except that acetonitrile gave almost the same color intensity as ethanol but its coloration was unstable. The PI-NCl reagent in ethanol was also stable for at least two weeks in the dark.

Under the established conditions, a calibration curve for terbutaline sulfate obeyed Beer's law over the concentration range from 0.7 to 270 μg/ml. Linear regression analysis of the absorbance *versus* the drug concentration gave a slope of 0.024, an intercept of 0.007, and a correlation coefficient, r, of 0.9999 ($n=24$). The relative standard deviations were 0.24% and 0.70% for 50 and 7 μg/ml of terbutaline sulfate ($n=10$), respectively.

Table I represents the analytical data of terbutaline sulfate in pharmaceutical preparations (tablets, injection, and fine granules) for the present method and the USP method. The data indicated that the recoveries of terbutaline sulfate from the dosage forms by both methods are in good agreement, but the present method is superior to the USP method in terms of the precision. The mean percent recoveries of spiked terbutaline sulfate in commercial dosage forms at 30% concentration of the labeled amounts obtained by the present method were $99.04 \pm 0.27\%$ ($n=5$) for injection, $99.9 \pm 0.36\%$ ($n=5$) for tablets and $101 \pm 0.48\%$ ($n=5$) for fine granules. The same lots assayed by the USP method gave mean percent recoveries of $97.0 \pm 1.14\%$ ($n=5$), $96.1 \pm 1.32\%$ ($n=5$), and $95.8 \pm 1.87\%$ ($n=5$), respectively.

As described in the previous paper,[15] the PI-NCl reagent does not produce any coloration with compounds such as amines, aldehydes, and ketones except that 1- and 2-naphthylamines give a positive result. In the present experiments, none of the commonly employed excipients, such as glucose, lactose, sucrose, starch, magnesium stearate, and calcium phosphate, interfered with the assay since they did not react with PI-NCl to give colored products.

Thus, the developed method can be successfully applied

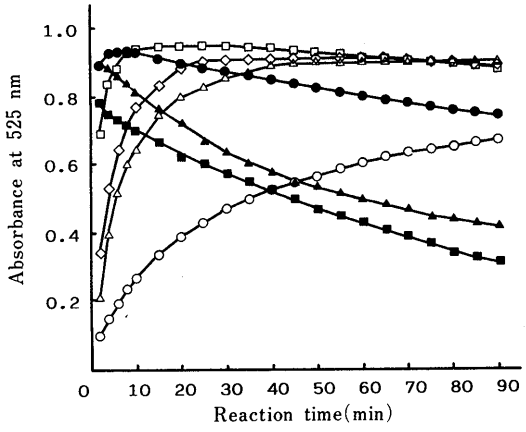

Fig. 1. Effect of pH and Reaction Time on the Color Development of Terbutaline Sulfate

To 0.25 ml of 200 μM terbutaline sulfate, 2 ml of 0.13 mg/ml PI-NCl ethanol solution and 1.5 ml of buffer were added. The absorbance was measured at 525 nm. ○, pH 7.5; △, pH 7.7; ◇, pH 8.0; □, pH 8.5; ●, pH 9.0; ▲, pH 11.0; ■, pH 13.0. Buffer: pH 7.5—9.0, 0.05 M boric acid–KCl/NaOH buffer; pH 11.0—13.0, 0.05 M KCl/NaOH buffer.

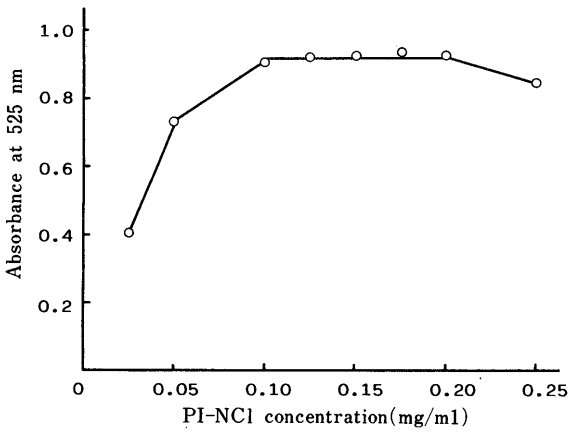

Fig. 2. Effect of PI-NCl Concentration on Color Development of Terbutaline Sulfate

Reaction conditions were the same as those for Fig. 1, except for the use of 0.05 M boric acid–KCl/NaOH buffer (pH 8.0). The absorbance was measured after standing for 40 min.

TABLE I. Determination of Terbutaline Sulfate in Pharmaceutical Preparations by the PI-NCl and USP Methods

Dosage form	Labeled amount	PI-NCl method		USP method	
		Found ± S.D.[a] (mg)	R.S.D. (%)	Found ± S.D.[a] (mg)	R.S.D. (%)
Injection	0.2 mg/ml	0.202 ± 0.001	0.36	0.209 ± 0.005	2.25
Tablets	2 mg/tablet	1.998 ± 0.009	0.44	2.008 ± 0.038	1.87
Fine granules	10 mg/g	9.938 ± 0.061	0.62	10.086 ± 0.297	2.94

a) Average of 5 experiments. S.D., standard deviation; R.S.D, relative standard deviation.

to the determination of terbutaline sulfate in pharmaceutical preparations with good recovery and precision. Under the proposed conditions, the apparent molar absorptivity obtained for terbutaline sulfate was 7.38×10^4 (3.69×10^4 as terbutaline) and this value is superior to those obtained for other color reagents such as MBTH–ferric chloride (5.06×10^4),[14] *p*-aminophenol–molecular oxygen (1.46×10^4),[13] 4-aminoantipyrine–potassium ferricyanide (1.10×10^4),[4] and 2,4-dibromoquinone chlorimide (1.31×10^4).[16]

The use of PI-NCl in alkaline solution at pH higher than 8 is also suitable for the determination of terbutaline by flow injection analysis (FIA) because of the faster reactivity.

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References

1) J. Bergman, H. Persson, and K. Wetterlin, *Experientia*, **25**, 899 (1969).
2) H. Persson and T. Olsson, *Acta Med. Scand. Suppl.*, **512**, 11 (1970).
3) "The Pharmacopeia of Japan, 11th ed.," Hirokawa, Tokyo, 1986, p. C-1702.
4) "The United States Pharmacopeia 21st, ed.," United States Pharmacopeial Convention, Rockville, MD, 1985, pp. 1019—1020.
5) L.-E. Edholm, B.-M. Kennedy, and S. Bergquist, *Chromatographia*, **16**, 341 (1982).
6) S. Bergquist and L.-E. Edholm, *J. Liq. Chromatogr.*, **6**, 559 (1983).
7) D. A. Williams, E. Y. Y. Fung, and D. W. Newton, *J. Pharm. Sci.*, **71**, 956 (1982).
8) V. D. Gupta, *J. Liq. Chromatogr.*, **9**, 1065 (1986).
9) R. A. Clarke, D. S. Davies, and T. A. Baillie, *Biomed. Mass Spectrom.*, **6**, 31 (1979).
10) S.-E. Jacobsson, S. Jonsson, C. Lindberg, and L.-A. Svensson, *Biomed. Mass Spectrom.*, **7**, 265 (1980).
11) M. M. Sethia, *Indian J. Pharm. Sci.*, **45**, 30 (1983).
12) K. G. Bhansali, *J. Pharm. Sci.*, **61**, 146 (1972).
13) K. E. Rao and C. S. P. Sastry, *Microchem. J.*, **32**, 293 (1985).
14) F. A. El-Yazbi, M. H. Abdel-Hay, and M. A. Korany, *Farm. Ed. Prat.*, **40**, 50 (1985).
15) S. Tanabe, C. Ise, T. Kosugi, and K. Kawanabe, *Anal. Sci.*, **5**, 43 (1989).
16) H. D. Gibbs, *J. Biol. Chem.*, **72**, 649 (1927).