Colorimetric Flow Injection Determination of Resorcinol-Type $\beta_2$-Adrenergic Drugs with Phenanthro[9,10-d]imidazole-2-N-chloroimide

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A simple and rapid flow injection method has been developed for the colorimetric determination of resorcinol-type $\beta_2$-adrenergic drugs: fenoterol hydrobromide, orciprenaline sulfate and terbutaline sulfate, by using phenanthro[9,10-d]imidazole-2-N-chloroimide (PI-NCI) as a color reagent. The color reaction between the drugs and PI-NCI occurs at alkaline pH. Under the optimized conditions, the calibration curves for the three drugs were linear over the range of 0.5–200 µM. The relative standard deviations (n=10) for fenoterol hydrobromide, orciprenaline sulfate and terbutaline sulfate were 1.7, 1.4 and 1.0%. The present method was applied to the determination of those drugs in commercial formulations.

Keywords Phenanthro[9,10-d]imidazole-2-N-chloroimide, fenoterol hydrobromide, orciprenaline sulfate, terbutaline sulfate, flow injection analysis, colorimetric determination, drug assay

Fenoterol hydrobromide [1-(3,5-dihydroxyphenyl)-2-[1-(4-hydroxybenzyl)ethylamino]ethanol hydrobromide], orciprenaline sulfate [1-(3,5-dihydroxyphenyl)-2-isopropylaminoethanol sulfate] and terbutaline sulfate [1-(3,5-dihydroxyphenyl)-2-t-butylaminoethanol sulfate], which have phenolic hydroxy groups in the meta positions, are widely used in the treatment of bronchial asthma. These drugs are commercially supplied as tablets, aerosol, syrup and injection.

The United States Pharmacopeia describes a colorimetric method for the determination of terbutaline sulfate with 4-aminoantipyrine and ferricyanide. However, the color product is not stable, so the measurements have to be carefully timed. Recently, colorimetric and fluorometric methods for the determination of terbutaline sulfate and orciprenaline sulfate have been reported. A reversed phase high performance liquid chromatographic method for the analysis of terbutaline sulfate has been also developed by using UV detector. However, these methods were inadequate for routine analysis because the operations were time-consuming. In a previous paper, we reported the synthesis and reactivity of phenanthro[9,10-d]imidazole-2-N-chloroimide (PI-NCI) as a new color reagent for phenolic compounds, and its application to the determination of terbutaline sulfate in pharmaceutical preparation. PI-NCI especially shows a strong coloration for 1,3-diphenols, 1-naphthol and dialkylphenols substituted in the 2- and 5- or 6-positions. It is also expected that PI-NCI will be a useful reagent for the sensitive detection and determination of resorcinol type drugs, because the molar absorptivity between this reagent and terbutaline sulfate has a higher value than those obtained in other color reagents so far reported.

This paper describes a sensitive, rapid and simple determination of fenoterol hydrobromide, orciprenaline sulfate and terbutaline sulfate in commercial formulations by flow injection analysis (FIA) using PI-NCI as a color reagent.

Experimental

Apparatus and FIA conditions

Figure 1 is a flow diagram of the FIA system, which consists of a double-plunger pump (DM2U, Sanuki Kogyo Co., Japan), a six port injection valve (10 µl, Shimamura Instrument Co., Tokyo, Japan), a spectrophotometric detector (L-4200 UV–VIS Detector, Hitachi Ltd., Tokyo, Japan), a dry reaction bath (Type DB-3, Shimamura Instrument Co.,) and an integrator (Chromatrope CR1B, Shimadzu Co., Kyoto, Japan). The reaction coil was Teflon tubing (1 m x 0.33 mm i.d.), which was set on the dry reaction bath at 60°C. The solutions both of 50 mM borate buffer (pH 11.5) as the carrier and of PI-NCI (30 mg/l) in ethanol as the reagent were delivered from a double-plunger pump at a flow rate of 0.8 ml/min. The colored products formed were monitored at 530 nm. The absorption signals were recorded by the peak height.
Materials

Terbutaline sulfate was supplied by Fujisawa Pharmaceutical Co. (Osaka, Japan). Fenoterol hydrobromide and orciprenaline sulfate were supplied by Boehringer Ingelheim Co. (Hyogo, Japan). All the dosage forms analyzed were from commercial lots. PI-NCl was synthesized by us. All other chemicals were of reagent grade.

Preparation of assay solution

Tablets: Weigh 10 tablets and then crush them thoroughly. Transfer an accurately weighed portion of the powder, equivalent to 5 mg of each drug, to a 50 ml volumetric flask, add 30 ml of 0.01 M hydrochloric acid, and shake by mechanical means for 10 min. Add 0.01 M hydrochloric acid to volume, mix and filter. The filtrate was diluted 10-fold with distilled water before analysis.

Fine granules: The fine granules (0.5 g) were weighed in a 25 ml volumetric flask; then the solution was made up to 25 ml with 0.01 M hydrochloric acid. The solution was diluted 20-fold with distilled water before analysis.

Syrup: Pipet syrup equivalent to 0.5 mg fenoterol hydrobromide from thoroughly shaken formulation into 50 ml volumetric flask, and dilute to volume with water.

Injection: Pipet solution equivalent to 0.2 mg terbutaline sulfate from thoroughly shaken injection into 20 ml volumetric flask and dilute to volume with distilled water.

Results and Discussion

The ethanol solution of PI-NCl was employed as a reagent by considering the reactivity and solubility. PI-NCl reacts with fenoterol hydrobromide, orciprenaline sulfate and terbutaline sulfate to form colored products having absorption maxima at 532, 529 and 530 nm at an alkaline pH. Since those maximum wavelengths were not changed in any alkaline pHs, the detection wavelength for the three drugs was set at 530 nm.

The effect of the concentration of the PI-NCl reagent on color formation of the standard drugs is shown in Fig. 2. Since the response for the three drugs remained constant in the concentration range of 25 - 40 mg/l PI-NCl, 30 mg/l of PI-NCl was chosen as the optimum concentration.

The color development between PI-NCl and the drugs is affected by the pH of the carrier. As shown in Fig. 3, the peak heights increased with rising pH and reached a maximum in the pH range of 11 to 12. Therefore, 50 mM borate buffer at pH 11.5 was used as the carrier.
Figure 4 shows the influences of the reaction time (coil length) and temperature on the color development for the three drugs. The color intensities from each drug increased with decreasing length of reaction coil, together with rising temperature. For coils longer than 3 m, which corresponded to the reaction time of 10 s, the color products of fenoterol and orciprenaline tended to decompose at the temperature higher than 30 - 40°C, whereas that of terbutaline increased up to 50°C. The maximal peak heights from each drug were obtained at 60°C with the coil length of 1 m, corresponding to the reaction time of 3 s. On increasing the reaction temperature beyond 60°C, small air bubbles appeared in the reaction coil.

In the flow system established, reproducible peak heights proportional to the concentrations of each drug were obtained. Calibration curves for fenoterol hydrobromide, orciprenaline sulfate and terbutaline sulfate were linear in the concentration range of 0.5 to 200 µM. The relative standard deviations obtained by injecting 10 samples for fenoterol hydrobromide, orciprenaline sulfate and terbutaline sulfate were 1.7, 1.4 and 1.0% at 10 µM each.

Table 1 shows the results of the recovery tests for the drugs added to each commercial formulation at the labeled amounts. The mean percent recoveries of each drug by the present FIA method were in the range...
99.6 - 102.1%, with the relative standard deviations of 0.36 - 1.47% (n=10).

The results of the determination of the three drugs in commercial formulations are summarized in Table 2. These data indicated that the FIA method described herein provides good recovery and precision data in all the dosage forms. The influences of the excipients, such as glucose, lactose, starch and sucrose, on the determination of drugs were investigated. None of them interfered with the measurement of drugs by the proposed FIA method.

In conclusion, fenoterol hydrobromide, orciprenaline sulfate and terbutaline sulfate in pharmaceutical preparations could be determined rapidly, sensitively and precisely by the FIA system using PI-NCl as a new color reagent. The present method permitted the analyses of at least 90 samples per hour. Therefore, the present FIA method is applicable for routine quality control of the three drugs in commercial formulations.

The authors are grateful to Fujisawa Pharm. Co. Ltd. and Böhringer Ingelheim Co. for the supply of reference standard.

References


(Received May 12, 1989)
(Accepted June 15, 1989)