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Note

Ion-pair high-performance liquid chromatographic method for the determination of atropine sulfate and tropic acid

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A recent review of the literature has indicated that much analytical work has been done on the quantification of atropine sulfate and some of its degradative products[1–7]. However, many of the methods used to analyze these compounds have remained basically unchanged over the years. In several cases, the procedures are arduous and time consuming. The sensitivity is limited. Many of the methods utilize colorimetric and spectrophotometric techniques for the determination of these tropanes in various types of therapeutic drugs. Quite frequently, several of the other belladonna derivatives react with the dyes and indicators used for color development. As such, the specificity is usually lacking for the described procedure.

Considering the difficulties of current methods, we have developed a rapid ion-pair high-performance liquid chromatographic (HPLC) method for quantifying atropine sulfate and tropic acid in therapeutic drugs. The method is sensitive and specific. Analysis time is 3.5 min per sample. No solvent extraction or pre-treatment is required prior to the determination.

As a rather simple analytical procedure, the described technique is applicable to a wide variety of drug formulations containing atropine sulfate and several of its degradative products.

EXPERIMENTAL*

Apparatus

The method was developed using a Waters Model ALC/GPC-204 liquid chromatograph, equipped with two Model 6000A high-pressure pumps, a 660 solvent programmer, a U6K loop injector, a 254 nm UV detector, a Houston Instrument Omni-Scribe A5000 dual-pen recorder and a Columbia Scientific Industries Supergrator-3-integrator.

Reagents

Spectroquality acetonitrile (Eastman-Kodak, Rochester, N.Y., U.S.A.) mixed with PIC B-7 reagent (1-heptane sulfonic acid, Waters Assoc., Milford, Mass., U.S.A.) was used as the mobile phase. Aqueous stock standard solutions (1 mg/ml) of atropine

* The manufacturers' names and products are given as scientific information and do not constitute an endorsement by the United States Government.

sulfate (U.S.P. reference standard, U.S.P.C. Inc., Rockville, Md., U.S.A.) and tropic acid (Aldrich, Milwaukee, Wisc., U.S.A.) were used to prepare all working standards. The hydrolysate samples were prepared by diluting the stock standard solution of atropine sulfate to 0.5 μg/μl and 0.6 μg/μl with 0.02 *N* HCl and 0.02 *N* NaOH.

Procedure

A 30 cm × 3.9 mm I.D. μBondapak C_{18} column (Waters Assoc.) was used to separate atropine sulfate and tropic acid. μBondapak C_{18} is a 10-μm particle size packing material, which is designed for both analytical and semi-preparative separations. The mobile phase consisted of 0.01 *M* 1-heptane sulfonic acid mixed with acetonitrile. The PIC B-7 reagent was prepared by mixing 20 ml of the pre-package reagent with 480 ml of glass distilled water. The pH of the solution was 3.40. A 35% solution of acetonitrile was mixed with 65% of the PIC B-7 reagent. Flow-rate for the dual pumping system was 1.5 ml/min. Column pressures ranged between 1200 and 1500 p.s.i. All separations were performed at ambient temperatures. A 2-μl sample was introduced onto the column through a continuous flow loop injector. The detection limit of the method for atropine sulfate was 200 ng on column. Peak areas were measured and calculated by an on-line computing integrator.

RESULTS AND DISCUSSION

Atropine sulfate, which is administered therapeutically as an anticholinergic and mydriatic agent, has gained much prominence over the years[8–11]. However, the development of simple and specific methods for analyzing this compound has failed to evolve with the popularity of the drug.

In 1973, Stutz *et al.*[12] utilized an HPLC method to separate and quantify atropine sulfate and a group of similar tropane alkaloids. In the interim, very few HPLC procedures have been reported in the literature.

As such, we have developed a simple ion-pair HPLC method for observing the fate of atropine sulfate when the drug is exposed to acids, bases and elevated temperatures. The method is also capable of quantifying tropic acid. Tropic acid, a major hydrolytic product of atropine is ineffective as a therapeutic modality.

In developing the analytical method for quantifying these two compounds, pure reference standards of atropine sulfate and tropic acid were chromatographed to determine the resolution and sensitity of the procedure. Fig. 1 represents the separation. From this initial run, all of the desired parameters for the method were met. Tropic acid was four times more sensitive in its quantifiability than atropine sulfate. A 50-ng sample of the compound was measured by the method. The resolution was also excellent for the two compounds. At the same time, a linear relationship was observed for atropine sulfate and tropic acid at the various absorbance ranges used in this study (0.005–0.02 *A*).

Data obtained from twenty analyses of an atropine sulfate anticholinergic preparation showed the method to be both accurate and precise. The concentration of atropine sulfate in a commercially prepared formulation, as established by our procedure was 0.51 mg/ml (standard deviation, ± 0.010; coefficient of variation, 1.96%). This value compared favorably with the manufacturers' value for the drug (0.52 mg/ml).

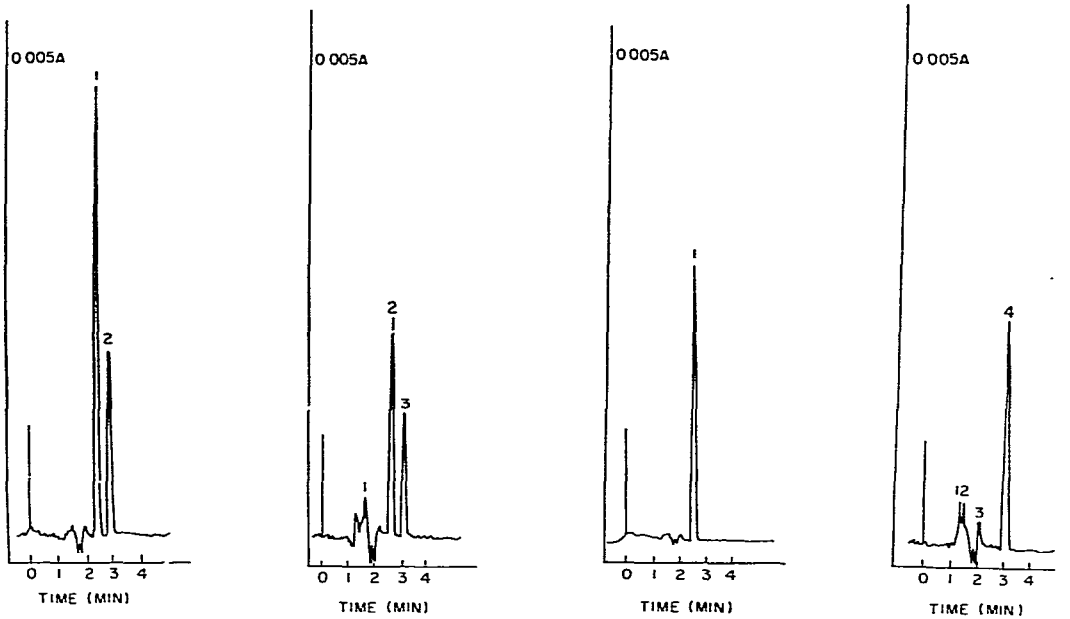

Fig. 1. Separation of a standard solution containing (1) 350 ng of tropic acid and (2) 1.0 μg of atropine sulfate. Column: 300 × 3.9 mm I.D. μ-Bondapak C_{18}. Mobile phase: 35% acetonitrile and 65% PIC reagent. Flow-rate, 1.5 ml/min. Column temperature 20°.

Fig. 2. A chromatogram of a 1.0-μg sample of atropine sulfate heated in 0.01 *N* NaOH at 37° for 60 min. 1 = Unknown; 2 = tropic acid; 3 = atropine sulfate.

Fig. 3. A chromatogram showing the conversion of atropine sulfate to tropic acid after 12 h of hydrolysis in 0.01 *N* NaOH at 37°.

Fig. 4. A chromatogram of a 1.2-μg sample of atropine sulfate heated in 0.01 *N* HCl at 37° for 60 min. 1, 2, 3 = Unknown; 4 = atropine sulfate.

Chromatographing the acidic and basic hydrolysates of atropine sulfate solutions produced the following chromatograms. Fig. 2 shows the formation of tropic acid after the atropine sulfate was heated at 37° in 0.01 *N* NaOH for 60 min; 15% of the atropine was hydrolyzed to the degradative product during this period. Incubating an aliquot of this partially hydrolyzed solution for an additional 11 h, converted the remaining atropine sulfate to tropic acid. The chromatogram shown in Fig. 3, depicts the results of this hydrolytic conversion.

Conversely, atropine sulfate heated in 0.01 *N* HCl at 37° for 60 min produced no measurable differences in the concentration of the drug (Fig. 4). Three small peaks were observed in the chromatogram, but their identities were not established.

From this study we were able to demonstrate an experimental model for the evaluation of a simple and specific analytical method for quantifying atropine sulfate and tropic acid. While the experiments we described should have obvious importance in probing the stability of atropine sulfate, our immediate objective in this study was to draw attention to the importance of ion-pair HPLC as a new and innovative analytical tool.

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