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Note

Determination of adiphenine hydrochloride and diphenylacetic acid by ion-pair high-performance liquid chromatography

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Adiphenine hydrochloride (2-diethylaminoethyl 2,2-diphenylacetate) is an antispasmodic and anticholinergic agent which has been used in clinical studies for the treatment of autonomic spastic dysfunctions occurring in smooth muscle of the gastrointestinal tract. The pharmacodynamic properties of this broad spectrum therapeutic drug were first reported in the literature 40 years ago[1]. Since then, a vast amount of research has been conducted in both man and animal exploring the therapeutic effectiveness of this compound in treating various disorders of the nervous system.

As an antagonist of acetylcholine in both the parasympathetic and central nervous systems of mammals, adiphenine hydrochloride is presently being used as a neuro-muscular blocking agent for offering protection against a variety of cholinergic compounds[2–3]. While these and other types of studies have attempted to define the therapeutic efficacy of this drug in treating pathologic and chemically induced neurological conditions, few methods have been developed which can readily quantify adiphenine hydrochloride and its degradation products without employing time consuming and tedious procedures.

In this paper, we described a simple, specific and sensitive reversed-phase high-performance liquid chromatographic (HPLC) method for separating and quantifying adiphenine hydrochloride and its principal degradation product, diphenylacetic acid. The procedure is applicable for determining each compound in simple and complex drug formulations. Amounts as low as 25 ng of either compound can be measured by the procedure. No pretreatment or derivatization is required prior to the analysis. The entire procedure requires 7 min per sample.

We are currently employing the method in our laboratory for studying the stability of adiphenine hydrochloride in drug formulations at various pH and thermal gradients.

EXPERIMENTAL*

Apparatus

The method was developed using a Waters Assoc. Model ALC/GPC-204

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

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liquid chromatograph (Milford, MA, U.S.A.), 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 (Burdick & Jackson Labs., Muskegon, MI, U.S.A.) mixed with PIC B-7 reagent (1-heptanesulfonic acid, Waters Assoc.) was used as the mobile phase. Standard solutions of diphenylacetic acid (0.5 μg/μl) were prepared from a reagent grade chemical (Pfaltz and Bauer, Stamford, CT, U.S.A.). Adiphenine hydrochloride was synthesized in our laboratory by reacting diethylaminoethanol with diphenylacetyl chloride in acetonitrile. It was purified by several crystallizations in acetonitrile. Identification was confirmed by its melting point (114–115°C), infrared and nuclear magnetic resonance spectroscopic properties.

Procedure

A 30 cm × 3.9 mm I.D. μBondapak C_{18} column (Waters Assoc.) was used to chromatograph adiphenine hydrochloride and diphenylacetic acid in standard solutions and experimental samples. The mobile phase consisted of equal volumes of acetonitrile and 0.01 *M* 1-heptanesulfonic acid. PIC B-7 reagent was prepared by dissolving 20 ml of the pre-packaged reagent into 480 ml of glass-distilled water. The pH of the solution was 3.40. A dual pumping system was used to deliver the 50–50% isocratic solution through the column. Flow-rate was 1.5 ml/min. All separations were performed at ambient temperatures. Samples were injected into the column through a continuous-flow loop injector. Peak areas were measured by an on-line computing integrator.

RESULTS AND DISCUSSION

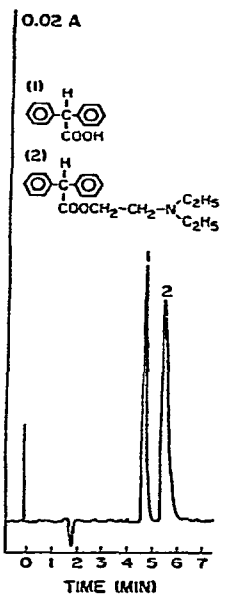
The systematic search for effective anticholinergic agents, offering maximum anticholinergic activity and minimum toxicity, as compared to atropine has been an effort involving a great deal of work[4,5]. Much of the renewed interest into this type of research has involved a family of drugs which complements the existing therapy presently available for treating various types of organophosphate poisonings[6]. Current research has focused on the diethylaminoethanol ester analogues.

In a series of recently published reports[7,8] we studied the degradative fate of these anticholinergic compounds upon their exposure to various pH and temperature conditions. The results of these studies were used to develop test procedures for evaluating the stability of these compounds under various packaging and storage conditions.

In order to observe the formation of the non-therapeutic oxidative by-products present in the antidotal preparations, sensitive and specific methods were required. For developing this new method for quantifying adiphenine hydrochloride and diphenylacetic acid, standard solutions and experimental samples were chromatographed. An example of the separation is shown in Fig. 1.

From a series of standards, ranging in concentration between 100 ng/μl to 1000 ng/μl, linearity was obtained for each compound. Correlation coefficients for adiphenine hydrochloride and diphenylacetic acid were 0.996 and 0.998, respectively.

At the same time, experimental samples of adiphenine hydrochloride were prepared in 0.01 *N* hydrochloric acid and 0.01 *N* sodium hydroxide. Each group was heated at 37°C for various time periods. The chromatograms below show the results of this study.



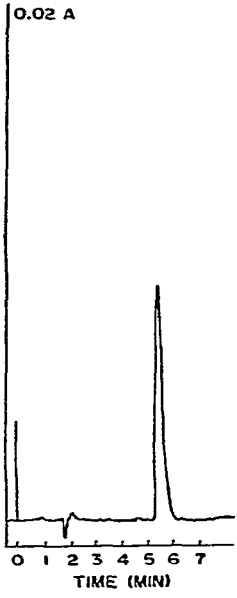


Fig. 1. Chromatogram of a standard solution containing (1) diphenylacetic acid (500 ng) and (2) adiphenine hydrochloride (800 ng). Conditions: column, 30 cm × 3.9 mm I.D. μBondapak C_{18}; mobile phase, acetonitrile–0.01 *M* 1-heptane sulfonic acid (1:1), pH 3.40; flow-rate, 1.5 ml/min; column temperature, ambient.

Fig. 2. Chromatogram of an 800 ng sample of adiphenine hydrochloride in 0.01 *N* sodium hydroxide at ambient temperature prior to heating at 37°C.

In assaying samples of the acid hydrolysates of adiphenine, no appreciable amount of degradation was observed at the different time periods used in this experiment. However, an opposite picture was seen for the alkaline hydrolysates. Hydrolytic breakdown occurred very rapidly at each reaction time. Fig. 2 represents an 800 ng sample of adiphenine hydrochloride at room temperature and at zero time. Upon incubating the samples at 37°C for times ranging from 30 sec to 2 min, more than 90% of the parent compound was degraded to its oxidative by-product (Figs. 3, 4 and 5). Although the same trend was noted with benactyzine and aprophen, we found adiphenine to be less stable than its two congeners.

From these and other studies, we have found that the maximum anticholinergic activity of the diethylaminoethanol esters is completely dependent upon maintaining a totally intact molecule. These data are supported by a recently published article[9] that states, "several chemical groups are needed for a molecule to have maximum anticholinergic activity: a quite large group containing a quaternary or (with smaller peripheral activity), a tertiary nitrogen atom, a phenyl or a similar group, a hydroxyl group and a cyclohexyl or similarly large lipophilic group".

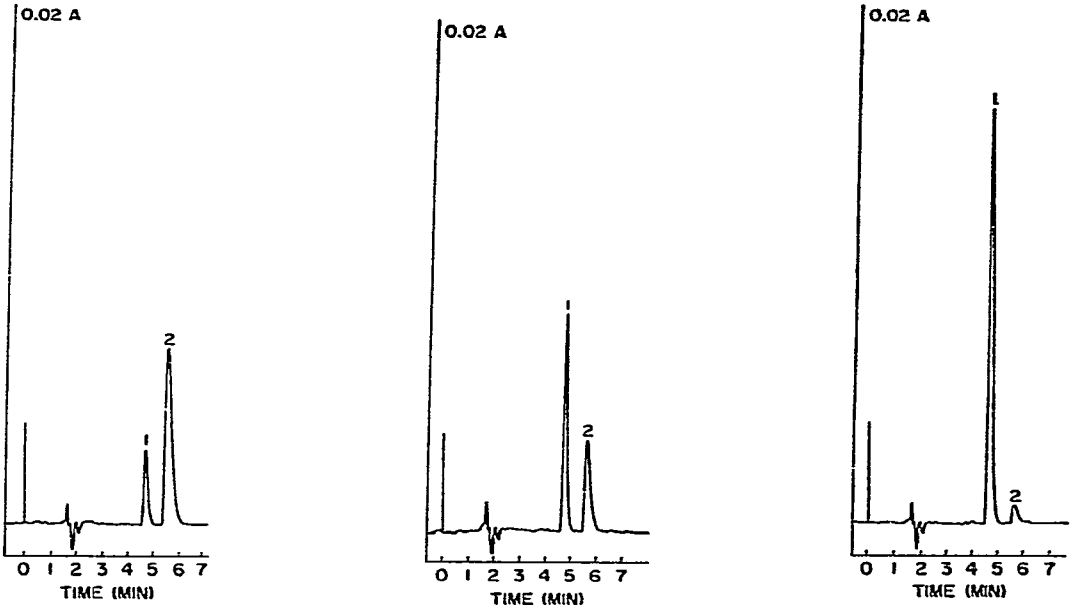


Fig. 3. Separation of (1) diphenylacetic acid and (2) adiphenine hydrochloride heated for 30 sec in 0.01 *N* sodium hydroxide.

Fig. 4. Chromatogram showing a 50% conversion of adiphenine hydrochloride (2) to diphenylacetic acid (1) after 60 sec.

Fig. 5. Chromatogram showing the hydrolysis of an 800 ng sample of adiphenine hydrochloride (2) to 750 ng of diphenylacetic acid (1) after 2 min.

Since specific chemical parameters are defined for maintaining an effective anticholinergic drug, any destablizing effect on the molecular structure of these chemical compounds will diminish or destroy the therapeutic effectiveness of these anticholinergic agents.

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