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## Note

# Determination of aprophen and its hydrolytic by-products by ion-pair high-performance liquid chromatography

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Aprophen (2-diethylaminoethyl 2,2-diphenylpropionate) is a synthetic analogue related to the diethylaminoethyl ester family. As such aprophen is administered prophylactically and therapeutically as an anticholinergic and antispasmodic agent[1]. Because of the ability of aprophen to penetrate the blood–brain barrier of mammals, it has been shown to produce high anticholinergic activity in both the central and peripheral nervous systems of animals.

In a series of comparative studies, where the anticholinergic activity of aprophen and some of its structural congeners were compared to atropine, the diethylaminoethyl ester analogues showed greater ganglion-blocking activity than atropine to protect mice poisoned with several types of nerve agents[2].

While these and other studies have done much to define the pharmacodynamic actions of aprophen in various biological systems, the advent of new high-performance liquid chromatographic (HPLC) techniques has also helped the researcher to understand better the chemicokinetic nature of these anticholinergic agents when they are subjected to various chemical and thermal conditions[3,4].

In this report, we describe a simple and specific ion-pair reversed-phase HPLC procedure for separating and quantifying aprophen and its principal degradation product, 2,2-diphenylpropionic acid. Amounts as low as 50 ng of each compound may be quantified by the method. No solvent extraction or pretreatment is required prior to the determination. Analysis time is 5 min per sample. The procedure is highly accurate and reproducible.

Because of the simplicity and specificity of the method, we are currently using the procedure in experimental studies to determine the stability and shelf life of aprophen in simple and complex drug formulations.

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## EXPERIMENTAL*

### *Apparatus*

A Waters Assoc. (Milford, MA, U.S.A.) Model ALC/GPC-204 liquid chromatograph was used to complete this study. The system consisted of two Model 6000A high-pressure pumps, a Model 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 Supergrator-3 integrator.

### *Reagents*

Spectroquality acetonitrile (Burdick & Jackson Labs., Muskegon, MI, U.S.A.) and PIC-B7 reagent (1-heptane sulfonic acid, Waters Assoc.) were used as the mobile phase. 2,2-Diphenylpropionic acid (Pfaltz & Bauer, Stamford, CT, U.S.A.) and synthesized aprophen were used to prepare the working standards (1 $\mu$g/$\mu$l). Aprophen was prepared in our laboratory by using the method of Zuagg and Horrom[5]. Its identification was confirmed by its melting point (161–162°C), infrared, nuclear magnetic resonance spectroscopic properties, and by chemical-ionization mass spectrometry.

### *Procedure*

A pre-packed 30 cm × 3.9 mm octadecylsilyl bonded-phase $\mu$Bondapak $C_{18}$ column (Waters Assoc.) was employed to chromatograph all compounds used in this study. The mobile phase consisted of 0.01 *M* 1-heptane sulfonic acid mixed with acetonitrile. PIC-B7 reagent was prepared by dissolving 20 ml of the pre-package reagent into 480 ml of glass distilled water. The pH of the solution was 3.40. A 75% solution of acetonitrile combined with 25% of the PIC-B7 reagent was isocratically pumped through the column using both pumps. Flow-rate was 1 ml/min. Column pressures ranged between 1000 and 1200 p.s.i. All separations were performed at ambient temperatures. Samples were introduced into the column through a continuous flow loop injector. The resulting peak areas were measured by an on-line computing integrator.

## RESULTS AND DISCUSSION

While much data have been compiled over the years describing the therapeutic values of aprophen in biological systems[6–9], very little information is available which describes a simple and specific method for observing the degradative fate of this compound while in storage or under experimental conditions.

From information obtained in a previous study[10], benactyzine, which is a chemical congener of aprophen was found to be highly unstable at elevated temperatures and pH values. It was also determined from these studies that the therapeutic efficacy of a benactyzine preparation used as an anticholinergic agent was diminished or completely lost due to chemical breakdown. Because of the structural similarities of benactyzine and aprophen, the use of ion-pair HPLC was reapplied to observe the

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

fate of the latter when it was experimentally subjected to thermal and chemical variations.

Chromatographing a series of aprophen and 2,2-diphenylpropionic acid samples, the sensitivity and accuracy of the method are demonstrated. Concentrations ranging 200 to 1000 ng/$\mu$l were used to plot the standard curve (Fig. 1). Each point on the from graph represents the average of five analyses. No individual value differed from its mean value by more than $\pm$ 2%. Linearity was observed for all absorbance ranges employed in this study (0.005–0.02 *A*).

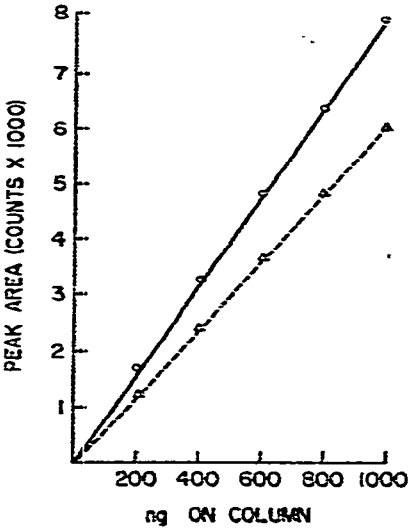


Fig. 1. Calibration curve of 2,2-diphenylpropionic acid (○) and aprophen (△) detected at 254 nm. Column: 300 × 3.9 mm I.D. $\mu$Bondapak $C_{18}$.

Concommitant with the analyses of the standard solutions, experimental samples of acid and alkaline hydrolysates of aprophen were also chromatographed. Solutions of aprophen were prepared in 0.01 *N* NaOH and 0.01 *N* HCl. Each group of samples was heated at 40°C in a controlled temperature water bath for various periods at time.

As was observed in the benactyzine studies, aprophen was relatively stable in 0.01 *N* HCl at 40°C for the different reaction times used in this study. However, the rate of degradation of aprophen occurred rapidly in 0.01 *N* NaOH at the elevated temperature. Hydrolytic breakdown of aprophen followed first order kinetics. The three chromatograms below show the rate of formation of 2,2-diphenylpropionic acid at various reaction times.

Fig. 2 represents the separation of an 800 ng sample of aprophen heated in 0.01 *N* NaOH for 1 min. During this short incubation time, the first signs of degradation appear. Small traces of 2,2-diphenylpropionic acid can be observed in the chromatogram. As the reaction times increased, greater amounts of the by-product were formed. Figs. 3 and 4 show the degree of degradation of aprophen after 5 and 60 min, respectively. After 1 h of heating, more than 60% of the aprophen was

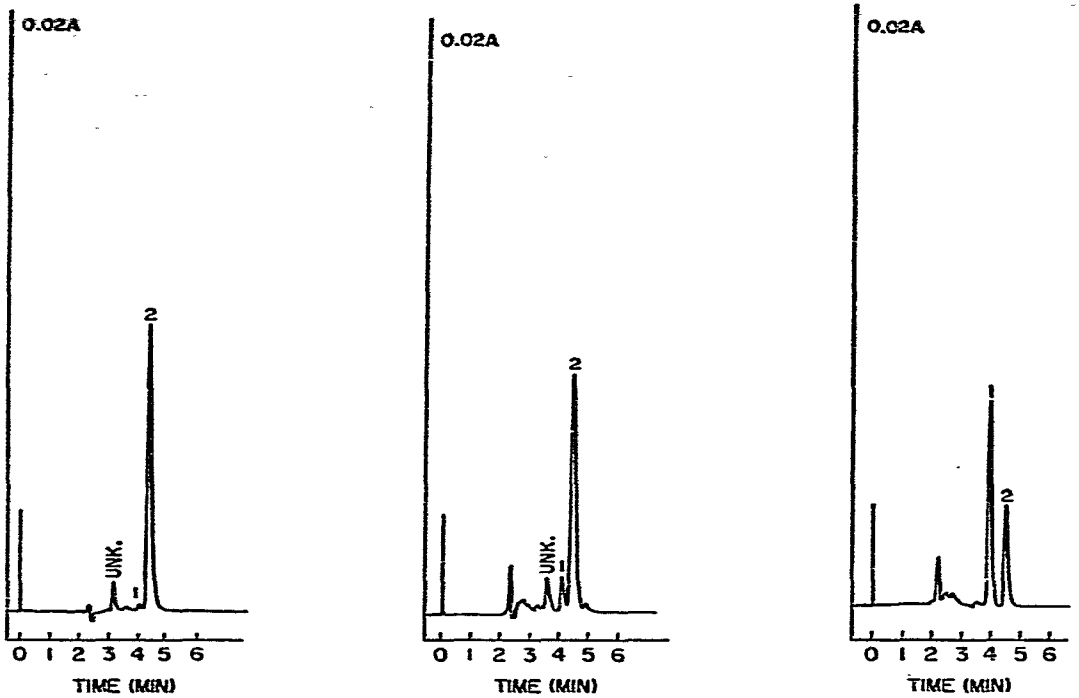


Fig. 2. Chromatogram of a 800-ng sample of aprophen heated in 0.01 *N* NaOH at 40°C for 60 sec. Peaks: 1 = 2,2-diphenylpropionic acid, 2 = aprophen.

Fig. 3. Separation of (1) 2,2-diphenylpropionic acid and (2) aprophen heated for 5 min in 0.01 *N* NaOH. Mobile phase: acetonitrile–PIC-B7 (75:25) reagent. Flow-rate, 1 ml/min. Column temperature: ambient.

Fig. 4. Chromatogram showing the conversion of aprophen to 2,2-diphenylpropionic acid after 1 h of hydrolysis in 0.01 *N* NaOH at 40°C.

degraded to 2,2-diphenylpropionic acid. Results obtained from a previous study (unpublished data) showed that the antidotal effectiveness of benactyzine was lost when 40% of the therapeutic formulation was broken-down to its oxidative by-product.

Since the collected evidence indicate that the stability of aprophen is dependent upon pH and temperature limitations, it is imperative that safeguards be taken to maintain the therapeutic efficacy of this anticholinergic drug by utilizing proper formulation and storage parameters.

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