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## Analysis of Meclofenoxate and Its Degradation Products by High Performance Liquid Chromatography<sup>1)</sup>

TOHORU TATSUHARA and FUMIE TABUCHI

Hospital Pharmacy, Tottori University School of Medicine<sup>2)</sup>

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An analytical method for meclofenoxate and its degradation products was established by high performance liquid chromatography. The chromatographic conditions were as follows: column,  $\mu$ -bondapak C<sub>18</sub>; mobile phase, MeOH—0.1% ammonium carbonate of pH 3.1; flow rate, 2.0 ml/min; spectrophotometric detection at 227 nm.

The mobile phase was found to be suitable for use as an extracting solvent for pharmaceutical preparations, since it caused almost no degradation of the sample, which has been reported to be very susceptible to hydrolysis.

**Keywords**—meclofenoxate; *p*-chlorphenoxyacetic acid; pharmaceutical analysis; high performance liquid chromatography; effect of methanol on hydrolysis

Meclofenoxate (I) has been used as a stimulant of the central nervous system. Grabowska<sup>3)</sup> and Yamana *et al.*<sup>4)</sup> studied the stability of I in aqueous solution and reported that it was hydrolyzed to *p*-chlorphenoxyacetic acid (II) and dimethylaminoethanol (III).

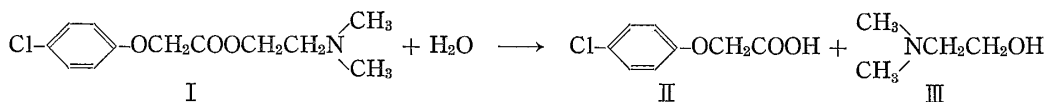


Chart 1

Some analytical methods for I, such as the dye method<sup>3)</sup> and the hydroxylamine method,<sup>4)</sup> have been reported. The dye method in the presence of large amounts of amino compounds, including the degradation product III, gives overestimated values. The hydroxylamine method is interfered with pharmaceutical additives such as phosphate. These methods cannot simultaneously estimate I and its degradation products.

This paper describes a high performance liquid chromatography (HPLC) procedure for the simultaneous assays of I and its degradation product II in pharmaceutical preparations.

### Experimental

**Apparatus**—A liquid chromatograph fitted with a septumless injector (Waters Assoc. Co., model U6K) and a variable wavelength spectrophotometer (Schoeffel Monitor SF770) was used. The detector was attenuated to 0.2 absorbance unit throughout at 227 nm. The column (4 × 300 mm), prepacked with  $\mu$ -Bondapak C<sub>18</sub>, was operated at ambient temperature with a mobile phase flow rate of 2.0 ml/min.

**Materials**—Meclofenoxate was recrystallized from iso-propyl alcohol. All other chemicals used were of guaranteed reagent grade.

**Mobile Phase**—Various mixtures of methanol and 0.1% ammonium carbonate were adjusted to a pH of 3.1 with 10% phosphoric acid and used as the mobile phase. The mobile phase was deaerated by ultrasonic irradiation at 400 KC under reduced pressure.

**Standard Solution**—Standard solutions of I and II were prepared by dissolving pure crystals in the solution used as the mobile phase at five different concentrations between 25 and 350  $\mu$ g/ml.

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- 3) I. Ggabowska, *Acta Polon. Pharm.*, **27**, 24 (1970).
- 4) T. Yamana, F. Ichimura, and K. Yokogawa, *Yakuzaigaku (The Archiv of Practical Pharmacy)*, **32**, 204 (1972).

**Determination of I and II in Standard Mixtures**—Appropriate quantities of accurately weighed I and II were added to one gram of a mixture of equal weights of corn starch and lactose, and mixed well. Next, 80 ml of the solution used as the mobile phase was added, and the whole was shaken by mechanical means for 15 minutes, and filtered through a Millipore filter (pore size; 0.65  $\mu\text{m}$ ) after dilution to 100 ml with the mobile phase. One milliliter of filtrate and 1 ml of an internal standard solution (benzoic acid 0.25 mg/ml in the solution used as the mobile phase) were pipetted into a 5 ml volumetric flask and diluted to the marked volume with the mobile phase, then analyzed by HPLC.

## Results and Discussion

Various methanol–buffer solutions of pH 3.1 were used as the mobile phase for HPLC, since I is labile in an alkaline or strongly acidic medium.<sup>4)</sup> When the ratio of methanol in the mobile phase was increased, the retention times of I and II became longer. The degradation product II could be conveniently separated from I using a mobile phase of methanol/0.1% ammonium carbonate (55/45: v/v). A typical chromatogram is shown in Fig. 1. Retention times of I and II were 2.2 and 4.3 minutes, respectively. III was not detected, because it has no absorption at 227 nm. After screening various compounds, such as salicylic acid, *o*-, *p*-dinitrobenzenes and phenol, as internal standards, benzoic acid was found to be suitable as shown in Fig. 1.

When a 5  $\mu\text{l}$  aliquot of the standard solution was injected into the column, the limits of detection for I and II were approximately 25  $\mu\text{g/ml}$ . The linear regression equations for calibration (concentration,  $x$ , versus peak height ratio,  $y$ ) were  $y_{\text{I}}=1.692 x_{\text{I}}+0.006$  (correlation coefficient of 0.998) for I, and  $y_{\text{II}}=0.731 x_{\text{II}}-0.010$  (correlation coefficient of 0.999) for II.

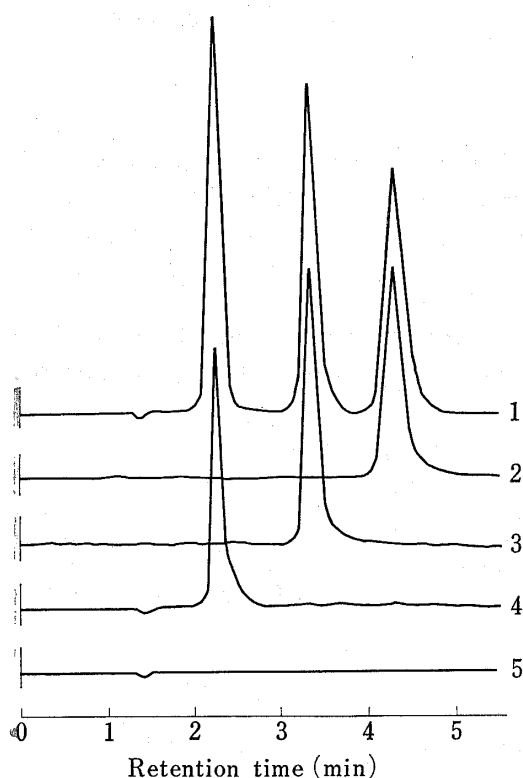


Fig. 1. Chromatogram obtained using Methanol-Ammonium Carbonate Buffer of pH 3.1 (55:45)

1, artificial mixture; 2, *p*-chlorophenoxyacetic acid; 3, benzoic acid (internal standard); 4, meclofenoxate; 5, water.

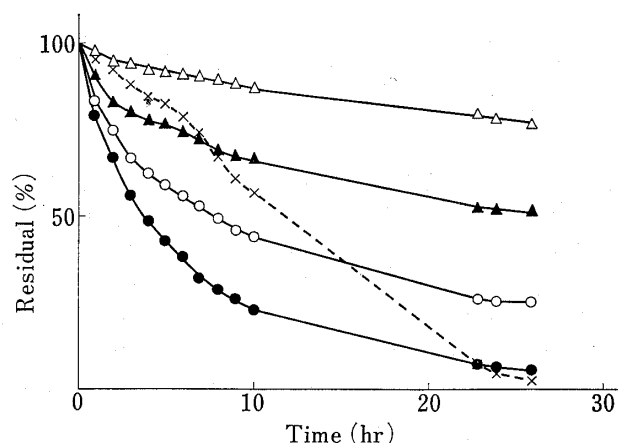


Fig. 2. Effect of Methanol on the Stability of Meclofenoxate

Concentration; 400  $\mu\text{g/ml}$ , temperature; 30°. Solvent:  $\Delta$ , water;  $\blacktriangle$ , 25% methanol;  $\circ$ , 50% methanol;  $\bullet$ , 75% methanol;  $\times$ , methanol.

TABLE I. Stability of Meclofenoxate at pH 3.1

Solvent	Initial concentration (mg/ml)	Residual (%)			
		1 hr	3 hr	6 hr	24 hr
Buffer	1.25	99.2	99.2	96.6	90.7
	12.5	100.9	99.1	96.5	91.2
MeOH-buffer (55:45)	1.25	100	99.1	98.3	89.6
	12.5	99.2	100.8	97.5	91.7

Temperature: 30°.

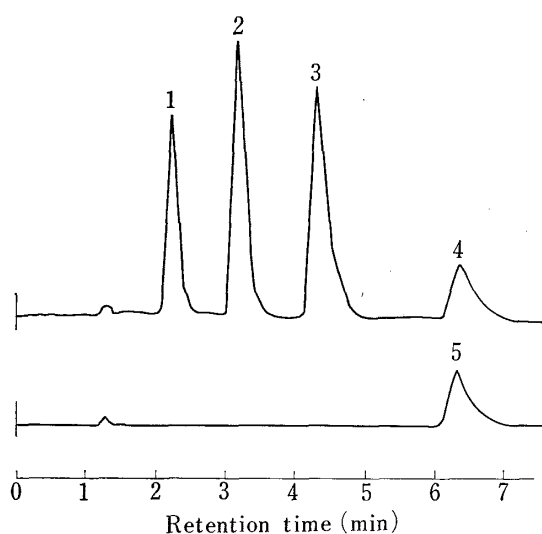


Fig. 3. Chromatogram of Meclofenoxate and Degradation Products in 55% Methanol

1, meclofenoxate; 2, benzoic Acid; 3, *p*-chlorophenoxyacetic acid; 4, unknown; 5, methyl *p*-chlorophenoxyacetate.

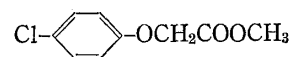


Chart 2

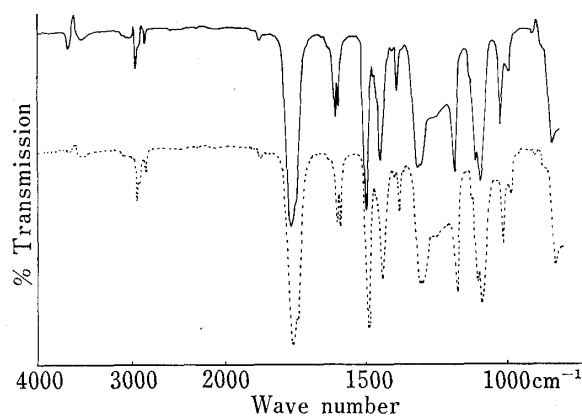


Fig. 4. IR Spectra (CHCl<sub>3</sub>)

—: methyl *p*-chlorophenoxyacetate,  
 ----: degradation product from meclofenoxate in MeOH.

Methanol, which readily dissolves I and II, is preferable to water as an extraction solvent for a sample containing degradation products, because II is only slightly soluble in water. However, I is more labile in methanol than in water, as shown in Fig. 2. The higher the methanol content in the methanol–water system, the more rapidly the degradation of I takes place (Fig. 2). As Fersht *et al.*<sup>5)</sup> and Nogami *et al.*<sup>6)</sup> reported for the hydrolysis of acetylsalicylic acid in a water–methanol system, it may be reasonable to explain this phenomenon on the basis of contact between the hydrophobic moiety of I and methanol, resulting in an increase of its affinity for water, accompanied by an increase of the hydrolysis rate of I. A difference between the rates of degradation of I in absolute methanol and in aqueous methanol may be due to another mechanism, since I was converted to methyl *p*-chlorophenoxyacetate (IV) in methanol. According to Yamana and his co-workers,<sup>4)</sup> I was most stable at pH 3.1. When buffer of pH 3.1 was employed in place of water in the above aqueous methanol, I was found to be stable, as shown in Table I.

When I was dissolved in mixtures of methanol and water and allowed to stand at room temperature for about 3 days, the chromatograms showed a new peak (Fig. 3). The retention time of this peak coincided with that of IV. This compound was isolated from these mixtures by HPLC and was identified as IV by infrared (IR) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy (in CDCl<sub>3</sub>)  $\delta$ : 3.80 (3H, singlet, –COOCH<sub>3</sub>), 4.61 (2H, singlet, –OCH<sub>2</sub>CO–), 6.83 (2H, doublet,  $J=9.5$  Hz, aromatic ring protons), 7.27 (2H, doublet,  $J=9.5$  Hz, aromatic ring protons), as shown in Fig. 4 and 5, respectively. Therefore, it appears that I was converted to IV in the presence of methanol.

On the basis of these results, the mobile phase for HPLC was also employed as an extracting solvent. I and II were dissolved as required in this solution, and no peak of IV was detected in the chromatogram. The analytical results for I and II from standard mixtures showed a good correlation between the calculated and found values for I and II, as shown in Table II. No interference by excipients in pharmaceutical formulations was found.

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