GLC Assay for Fenclorac

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Abstract □ A rapid stability-indicating GLC method is described for the determination of fenclorac as the diethylamine salt in dosage formulations. The procedure is also applied in detecting and quantitating the weight percent of 3-chloro-4-cyclohexylphenylglycolic acid and α-chloro-4-cyclohexylphenylacetic acid impurities associated with the synthesis of the fenclorac drug substance. The former compound, in addition to being an impurity, is also the degradation product of fenclorac. The procedure involves preparation of the silyl derivatives, addition of an internal standard (triphenylethylene), and use of a hydrogen flame-ionization detector. The column, with a phenyl methyl silicone liquid phase and operated at about 195°, is well suited for the separation. The silyl derivatives were characterized by GLC–mass spectrometry.

Keyphrases □ Fenclorac—GLC analysis, pharmaceutical dosage formulations □ GLC—analysis, fenclorac in pharmaceutical dosage formulations □ Anti-inflammatory agents—fenclorac, GLC analysis in pharmaceutical dosage formulations

Anti-inflammatory properties have been attributed (1) to aryl- and alkyl-substituted phenoxyacetic acids. Recently, a U.S. patent (2) was issued for a series of substituted phenylacetic acid compounds having high anti-inflammatory, analgesic, and antipyretic activities and low toxicity. Preferred compounds of this series are fenclorac (α,3-dichloro-4-cyclohexylphenylacetic acid) and its esters and salts. The pharmacological activity of fenclorac diethylamine (I) has been investigated[1].

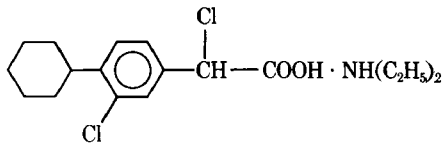
This publication is one of a series designed to provide information concerning the chemical kinetics[2] and analytical chemistry of I. A procedure is described for the determination of I in pharmaceutical dosage forms. The procedure is also applied in detecting and quantitating the impurities associated with the synthesis of I. These impurities are 3-chloro-4-cyclohexylphenylglycolic acid (II) and the diethylamine salt of α-chloro-4-cyclohexylphenylacetic acid (III). Compound II, in addition to being an impurity, is also the degradation product of I.

The developed GLC method requires little sample manipulations. Sample preparation is restricted solely to a simple solution of the drug and a silylation reaction with *N,O*-bis(trimethylsilyl)acetamide[3], necessitating approximately 15 min for completion.

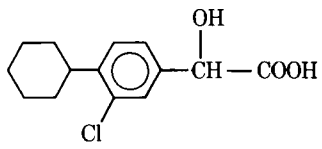
This procedure offers several advantages over derivatizing reagents such as diazomethane or boron trifluoride. Diazomethane is toxic, potentially explosive, and rather difficult to prepare and use. Boron trifluoride in methanol can promote side reactions through hydrolysis of labile compounds and complicate quantitation.

EXPERIMENTAL

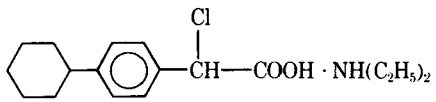
Instrumentation—A gas chromatograph[4] and electronic integrator[5], equipped with a flame-ionization detector and glass column [1.2 m (4 ft) × 2 mm i.d., packed with 5% phenyl methyl silicone[6] on 80–100-mesh silanized diatomaceous earth[7]], were used. The operating temperatures were: column, 195 or 185°; detector, 235°; and injection port, 235°. Nitrogen, with a flow rate of 30 ml/min, was the carrier gas.



I



II



III

Materials—Methylene chloride[8] and triphenylethylene[9] were used without further purification. *N,O*-Bis(trimethylsilyl)acetamide was used as the silylating reagent.

Assay of I in Dosage Formulations—*Internal Standard Preparation*—Triphenylethylene (125 mg) was transferred to a 50-ml volumetric flask and dissolved in and diluted to volume with methylene chloride.

Reference Standard Preparation— Reference I (125 mg) was accurately weighed into a 50-ml volumetric flask and dissolved in and diluted to volume with methylene chloride. This solution was prepared fresh daily.

Sample Preparation—A suitable quantity of tablets or the contents of capsules, after determining the average weight, was transferred to a mortar and pestle and ground to a fine powder. The equivalent of 125 mg of I was accurately weighed into a 50-ml volumetric flask, and about 30 ml of methylene chloride was added. The flask was stoppered and shaken vigorously for about 5 min, and the solution was diluted to volume with methylene chloride. A portion of the solution was centrifuged in a screw-capped[10] test tube to clear it of insoluble formulation excipients.

Two-milliliter aliquots of the sample and reference standard preparations, equivalent to 5.0 mg of I, were transferred to respective glass vials. To each vial were added 1.0 ml of the internal standard preparation and 0.20 ml of *N,O*-bis(trimethylsilyl)acetamide. The vials were securely closed with polyethylene stoppers, shaken vigorously, and allowed to stand at room temperature for not less than 15 min.

Procedure—The chromatographic column was preconditioned with several injections of the derivatized standard solution prior to analysis. Two microliters of this solution was injected until a constant peak area ratio of I to the internal standard was obtained; then 2 μl of the derivatized sample solution was injected in duplicate.

[1] Nuss *et al.*, to be published.
[2] C. M. Won *et al.*, to be published.
[3] Pierce Chemical Co., Rockford, Ill.
[4] Hewlett-Packard 7620A.
[5] Hewlett-Packard 3370A.
[6] OV-25, Analabs, Inc., Northhaven, Conn.
[7] Chromosorb W-H. P., Celite Division, Johns-Manville Products Corp.
[8] GC spectrophotometric grade, J. T. Baker Co.
[9] Eastman Kodak Co.
[10] Lined with Teflon (du Pont).

Table I—Response Factors and Relative Retention Time

Compound	Response Factor	Relative Retention
I	1.00	1.00
II	1.42	0.76
III	1.02	0.67

Calculations—The I content in pharmaceutical dosage forms was calculated as follows:

$$\text{mg of I/dosage unit} = \frac{(R_u)(C_s)(A.W.)}{(R_s)(W)} \quad \text{(Eq. 1)}$$

where R_u and R_s are the peak area ratios of I to the internal standard for sample and standard solutions, respectively; C_s is the milligrams of I in the reference standard preparation; $A.W.$ is the average dosage weight; and W is the weight of sample taken in the sample preparation.

Assay of Related Impurities in I Drug Substance—*Instrumentation*—The instrumentation was the same as previously described, except that the column operating temperature was lowered to 185°.

Determination of Response Factors—Two milligrams each of I, II, and III were accurately weighed and combined in a glass vial. To the vial were added 1.0 ml of methylene chloride and 0.20 ml of *N,O*-bis(trimethylsilyl)acetamide. The vial was securely closed with a polyethylene stopper, shaken vigorously, and allowed to stand at room temperature for not less than 15 min.

The chromatographic column was preconditioned with several injections of the standard mixture prior to analysis; then 2 μl of this mixture was injected in duplicate. The response factors of II and III relative to I were determined using the following sample calculations:

$$\text{specific response for II} = \frac{\text{peak area of II}}{\text{mg of II in mixture}} \quad \text{(Eq. 2)}$$

and:

$$\text{response factor for II} = \frac{\text{specific response for II}}{\text{specific response for I}} \quad \text{(Eq. 3)}$$

Typical response factors and retention times relative to I are shown in Table I. A chromatogram is given in Fig. 1 for a typical mixture.

Sample Preparation—Ten milligrams of I drug substance was transferred to a glass vial, and 1.0 ml of methylene chloride and 0.20 ml of *N,O*-bis(trimethylsilyl)acetamide were added. The vial was securely closed with a polyethylene stopper, shaken vigorously, and allowed to stand at room temperature for not less than 15 min. Two microliters of the sample preparation was injected, and the peak areas of each impurity, if any, and I were recorded. The peak area of each impurity was normalized by dividing by its respective response factor calculated previously.

Calculations—The weight percent of an impurity was calculated as follows:

$$\%\ \text{impurity} = \frac{\text{normalized peak area of impurity} \times 100}{\text{sum of all normalized peak areas in chromatogram}} \quad \text{(Eq. 4)}$$

RESULTS AND DISCUSSION

Derivatization Reagent—The use of *N,O*-bis(trimethylsilyl)-acetamide as the derivatizing reagent was prompted by the failure of boron trifluoride in methanol to form a stable derivative. Under the conditions of esterification, which required the application of heat to catalyze the reaction, partial decomposition of I to II occurred, resulting in a mixture of the methyl esters. With *N,O*-bis(trimethylsilyl)acetamide, derivatization was complete at room temperature within 2 min and the derivatives were stable up to 8 hr.

Table II—Fragmentation Pattern in Mass Spectral Examination of the Trimethylsilyl Derivative of I

Mass *m/e*	Fragment Ion
358	Molecular ion (M)
343	$M - CH_3$
314	$M - CO_2$
260	$M - [CH_3 + C_6H_{11}]$

Table III—Fragmentation Pattern in Mass Spectral Examination of the Trimethylsilyl Derivative of II

Mass *m/e*	Fragment Ion
412	Molecular ion (M), not observed
397	$M - CH_3$
369	$M - [CH_3 + CO]$
295	$M - [CH_3 + CO + OSi^+(CH_3)_2]$

Peak Characterization—GLC–mass spectrometry data[11] indicated the molecular ion of the derivative of I to be at *m/e* 358, corresponding to the monotrimethylsilyl derivative. The molecular ion of the derivative of II was not observed at *m/e* 412; however, an ion fragment found at *m/e* 397 was considered to be the disubstituted trimethylsilyl derivative minus one methyl group. This finding confirmed the formation of the ditrimethylsilyl derivative.

GLC–mass spectrometry was not performed on III due to its structural similarity to I, having only one active hydrogen capable of being silylated. The fragmentation pattern of the mass spectral examination of the trimethylsilyl derivatives of I and II are listed in Tables II and III, respectively.

Column Conditioning—Earlier work indicated that preconditioning of the GLC column was necessary to prevent excessive adsorption and peak loss of the trimethylsilyl derivatives of I and III. These phenomena did not occur with II. Apparently, the α-chlorine of I and III, which is of a labile nature, combined with active sites on the column support or glass column wall. Various solid supports were tried without success. Removal of the silanized glass wool plug at the head of the column did not rectify the problem.

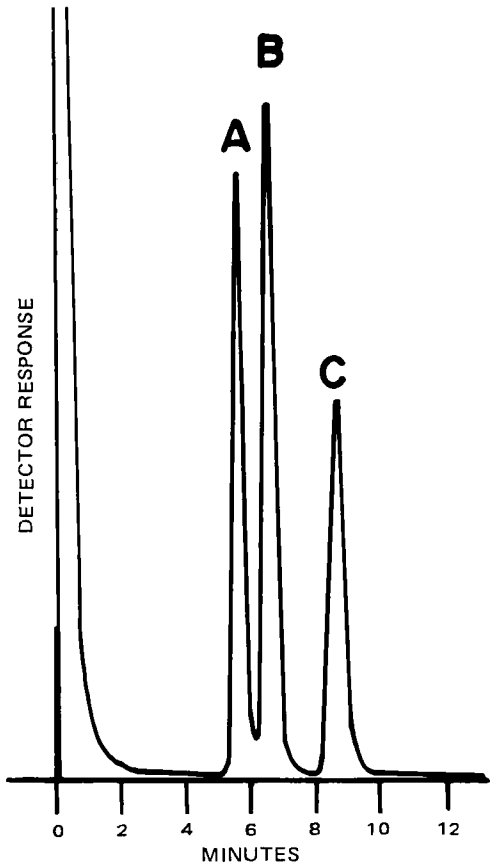


Figure 1—*Gas chromatogram of the trimethylsilyl derivatives of a mixture (prepared in the determination of response factors) containing III (A), II (B), and I (C).*

[11] Analysis performed by Analytical Development Corp., Monument, CO 80132

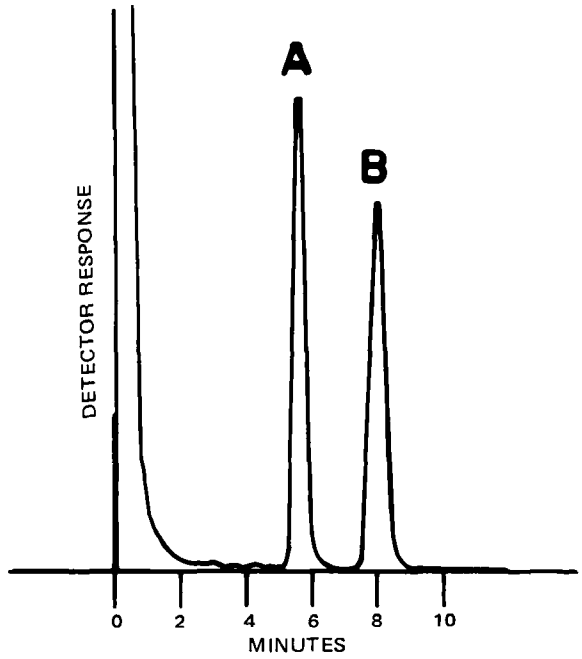


Figure 2—*Gas chromatogram of a sample preparation containing I as the trimethylsilyl derivative (A) and triphenylethylene, the internal standard (B).*

Preconditioning was a necessity when columns were initially packed; it was then only necessary to condition the column at normal operating conditions overnight before analyses were performed. The following preconditioning procedure was employed: 150° for 1 hr with a 30-ml/min carrier gas flow, 250° for 2 hr with no carrier gas flow followed by rapid cooling to room temperature, and 195° for 50–60 hr with a 30-ml/min carrier gas flow.

Table IV—Recovery Data

Mixture	I Added, mg	I Recovered, mg	Recovery, %
A	80.1	75.8	94.6
B	101.3	102.0	100.7
C	125.2	126.4	101.0
D	149.0	153.8	103.2
E	176.0	184.6	104.9

Table V—Statistical Evaluation

Determinations	I, mg
1	128.2
2	130.0
3	127.8
4	129.3
5	129.6
6	128.9
Mean	129.0
SD	±0.84
Precision, %	±0.65

Linearity—The detector response for I was linear in the 0.31–3.13-mg/ml range at a constant internal standard concentration. Column adsorption of I was indicated by the linear plot intersecting the concentration axis. By saturating the column with multiple sample injections until a constant peak area ratio of I to the internal standard was obtained, the adsorption phenomenon was kept relatively constant.

A minimum detectable quantity of II and III in the I drug substance was 0.01%.

Recovery—A study was undertaken to estimate the recovery of I from a synthetic mixture containing some common pharmaceutical excipients. Various amounts of I were added to a placebo mixture containing starch, lactose, microcrystalline cellulose, magnesium stearate, dibasic calcium phosphate, colloidal silicon dioxide, and hydroxypropyl cellulose. The mixtures were contained in 50-ml volumetric flasks, and the I content was assayed according to the procedure for dosage formulations. Results indicated essentially complete recovery (Fig. 2 and Table IV).

Precision—A statistical evaluation of the precision of the method was performed by making replicate injections of a placebo mixture containing I (Table V).

REFERENCES

(1) B. J. Northover, *J. Pathol. Bacteriol.*, **85**, 361(1963).

(2) J. Diamond and N. J. Santora, U.S. pat. 3,864,384 (Feb. 4, 1975).

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