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Short communication

# Determination of enantiomeric excess of α-hydroxy-3-phenoxybenzeneacetonitrile and its *n*-butyl ester by chiral high-performance liquid chromatography☆

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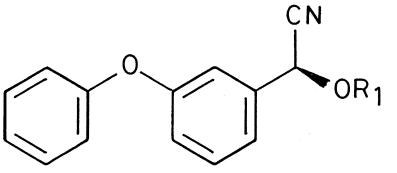
**Abstract**

Determination of enantiomeric excess of α-hydroxy-3-phenoxybenzeneacetonitrile, an important intermediate in the production of several pyrethroid insecticides, is usually done after derivatization and gas chromatographic analysis on a β-cyclodextrin-based column. In this communication we report a direct determination of enantiomeric excess of α-hydroxy-3-phenoxybenzeneacetonitrile and its *n*-butyl ester by chiral HPLC on Chiralcel OJ (Daicel, Japan) in a single run without derivatization. 

*Keywords:* Enantiomeric excess; α-Hydroxy-3-phenoxybenzeneacetonitrile; Cyanohydrins

## 1. Introduction

Synthesis of the cyanohydrin, (*S*)-α-hydroxy-3-phenoxybenzeneacetonitrile **5** (Fig. 1), is an industrially important process since it is used in the production of several pyrethriod insecticides such as Flucythrinate, Esfenvalerate, Cypermethrine and Deltamethrine. In the manufacturing process, the cyanohydrin **5** is prepared by hydrolysis or transesterification of the corresponding ester **1** by an enzyme such as lipase from *Arthrobacter* sp. or *Pseudomonas cepacia* [1–3] or by addition of HCN to 3-phenoxybenzaldehyde in the presence of the enzyme oxynitrilase [4]. Known analytical procedures for determination of the enantiomeric excess (e.e.) of **5** are mostly based on derivatization followed by either gas chromatographic (GC) or high-performance liquid chromatography (HPLC) analysis. These methods include derivatization of the




(R,S)-1 ($R_1$ = $COCH_3$ or $COCH_2CH_2CH_3$)
(S)-2 ($R_1$ = $COCH_2CH_2CH_3$), (R)-3 ($R_1$ = H)
(R)-4 ($R_1$ = $COCH_2CH_2CH_3$), (S)-5 ($R_1$ = H)

Fig. 1. Structures of the cyanohydrins.

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cyanohydrin to an acetate followed by chiral GC on β-cyclodextrin [5]; derivatization with (+)-methoxytrifluoromethyl phenylacetic acid (MTPA) chloride and the analysis of the derivative by $^{19}$F-nuclear magnetic resonance (NMR) [6] or $^{1}$H-NMR [7], or by HPLC [8]; preparation of *tert*.-butyldimethylsilyl derivative followed by chiral HPLC analysis [9] or derivatization with (*S*)-naproxen followed by analysis by reversed-phase HPLC [10]. Determination of the enantiomeric excess of the cyanohydrin can also be done by $^{1}$H-NMR using a chiral shift reagent such as tris-[3-(heptafluoropropylhydroxymethylene)-d-camphorato]europiumIII [Eu(hfc)$_3$] [11]. All these methods are tedious and time consuming. Besides, the cyanohydrins are prone to decomposition to corresponding aldehydes and racemization in the presence of a base during derivatization. Considering the industrial importance of the (*S*)-cyanohydrin **5** which is needed in excess of 1000 tonnes per annum, the present work was prompted by the need to develop a suitable analytical tool for determination of the enantiomeric excess of **5** which is reliable and simple for routine analysis of the enzyme catalyzed hydrolysis/transesterification reaction. Herein we report a simple technique for a direct determination of the enantiomeric excess of cyanohydrin **5** by chiral HPLC where the reaction mixture after proper dilution can be directly injected onto the column for the analysis where the peaks for the (*R*)- and (*S*)-cyanohydrin are well resolved. Although the reported substrate for the enzymatic preparation of **5** is the acetate **1** (R=COCH$_3$) [1–3], we have found that the corresponding butyrate ester **1** [R=CO(CH$_2$)$_2$ CH$_3$] is equally useful [11] with the added advantage that the peaks for the (*R*)- and (*S*)-esters are also well resolved to allow determination of the enantiomeric excess of unreacted ester concomitantly with that of the product cyanohydrin.

The procedure described herein also allows for the determination of the extent of cyanohydrin decomposition to aldehyde to obtain a complete analytical profile of the reaction mixture.

## 2. Experimental

### 2.1. Apparatus

HPLC analysis was done on Hewlett-Packard HP1090 HPLC unit equipped with diode array detector and HP Chemstation software, and fitted with a chiral HPLC column, Chiralcel OJ, of 250×5 mm obtained from Daicel, Japan. Optical rotations were measured on a Jasco DIP 270 polarimeter.

### 2.2. Reagents

HPLC-grade 2-propanol and *n*-hexane were purchased from Qualigens Fine Chemicals, India. Lipase [EC. 3.1.1.3] from *Pseudomonas cepacia* was obtained from Fluka, Switzerland while *Candida rugosa* lipase was obtained from Sigma, USA.

### 2.3. Preparation of the racemic acetate and butyrate esters *1* of α-hydroxy-3-phenoxybenzeneacetonitrile

The racemic acetate ester was prepared by reaction of 3-phenoxybenzaldehyde and acetic anhydride in a biphasic system of 1,2-dichloroethane and water in the presence of benzyltriethylammonium chloride as phase transfer catalyst [2]. The *n*-butyrate ester was also prepared by a similar strategy using *n*-butyryl chloride instead of acetic anhydride.

### 2.4. Preparation of (R)-α-hydroxy-3-phenoxybenzeneacetonitrile *3* and (S)-α-hydroxy-3-phenoxybenzeneacetonitrile *5*

The (*S*)-cyanohydrin **5** (e.e.>99%) and (*R*)-butyrate **4** (e.e. 96%) were prepared by transesterification of the racemic butyrate with *n*-butanol catalyzed by *Pseudomonas cepacia* lipase immobilized on celite as described in the literature [3]. The (*S*)-cyanohydrin **5** (e.e.>99%) was also prepared separately by a similar strategy using the acetate ester instead of the butyrate ester. The product (*S*)-cyanohydrin **5** obtained by both routes was identical. The (*R*)-cyanohydrin (e.e. 73.1%) was prepared by hydrolysis of the racemic acetate **1** with *Candida rugosa* lipase in acetate buffer (pH 4.5) up to 50% conversion [12] and the corresponding unreacted (*S*)-acetate ester was also obtained in the same reaction. All the products were purified by column chromatography [2] and characterized by $^{1}$H-NMR. The absolute stereochemistry was assigned on measurement of optical rotation and comparison with literature values. (*S*)-Butyrate ester **2** $[\alpha]_D^{25}$ 3.38 (*c* 1,

chloroform), e.e. 67.5%; (*R*)-cyanohydrin **3** $[\alpha]_D^{25}$ 17.8 (*c* 1, chloroform), e.e. 73.1%; (*R*)-butyrate ester **4** $[\alpha]_D^{25}$ −4.9 (*c* 1, chloroform) e.e. 96%, literature value for corresponding (*R*)-acetate −7.02 (*c* 1, chloroform) [2]. (*S*)-Cyanohydrin **5** $[\alpha]_D^{25}$ −24.3 (*c* 1.2, chloroform), e.e.>99%; literature $[\alpha]_D^{25}$ −23.4 (*c* 1.2, chloroform), e.e. 96% [2].

## 3. Results and discussion

### 3.1. Analytical conditions

Most of the earlier reports on the enzymatic preparation of (*S*)-cyanohydrin **5** are based on hydrolysis of the corresponding acetate **1** ($R_1$=$CH_3$). Several attempts were hence made to resolve the racemic acetate on the chiral HPLC columns Chiralcel OD and Chiralcel OJ by changing the solvent composition and flow-rates without any success. Although the acetate ester eluted as a single peak at 32.1 min, it was possible to separate the (*R*)- and (*S*)-cyanohydrins **3** and **5** on Chiralcel OJ with an excellent resolution with retention times 18.2 and 22.7 min, respectively. Under the analytical conditions used for the resolution, the acetate ester eluted much later at 32.1 min to allow determination of the enantiomeric excess of the cyanohydrin. These results were still somewhat unsatisfactory since it is desirable to follow the course of reaction by measuring the decrease in concentration of the (*R*)- or (*S*)-ester depending on the enzyme used.

We then explored the possibility of using the butyrate ester instead of the acetate ester. Both the enantiomers of the *n*-butyryl ester and the cyanohydrins were well separated on the Chiralcel OJ column (Daicel) in the same run, and it was very convenient to follow the course of reaction by chiral HPLC. As seen from Fig. 2 all the constituents of the reaction mixture including 3-phenoxybenzaldehyde are well resolved. This overcomes the tedium of derivatization of the cyanohydrin and also gives a complete picture as the reaction proceeds.

The optimized conditions for chiral HPLC analysis are as follows: column Chiralcel OJ, 205×5 mm (Daicel); mobile phase 18% 2-propanol in *n*-hexane; flow-rate of 0.7 ml/min and detection wavelength 240 nm. Under these conditions retention times are:

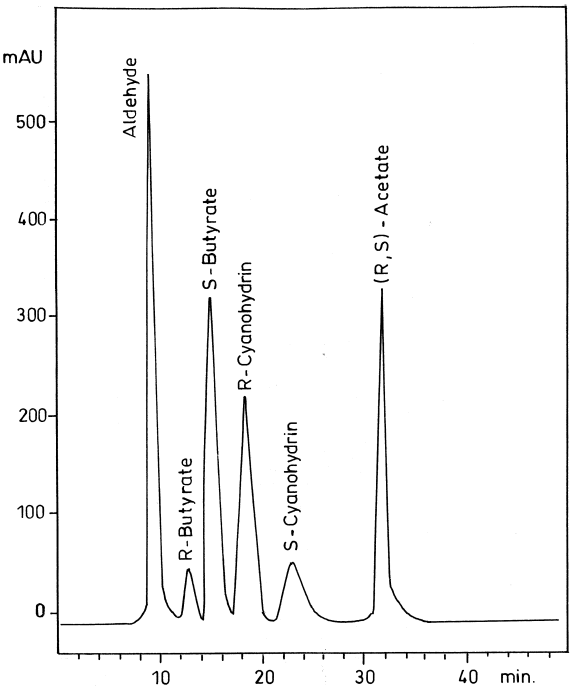


Fig. 2. HPLC chromatogram showing separation of (*R*)- and (*S*)-enantiomers of α-hydroxy-3-phenoxybenzeneacetonitrile and of the corresponding buyrate and acetate esters on the Chiralcel OJ 250×5 mm chiral HPLC column (Daicel). Mobile phase 18% 2-propanol in *n*-hexane; flow-rate, 0.7 ml/min and detection wavelength 240 nm.

aldehyde 9.2; (*R*)-butyrate **4** 12.7; (*S*)-butyrate **2** 14.9; (*R*)-cyanohydrin **3** 18.2; (*S*)-cyanohydrin **5** 22.7; (*R*,*S*)-acetate **1** 32.1 min.

The utility of the method is exemplified in Fig. 3 which shows the course of the reaction for transesterification of the racemic butyrate ester with *n*-butanol catalyzed by *Pseudomonas cepacia* lipase immobilized on celite in *n*-hexane.

In another application, the racemic acetate ester **1** (R=$COCH_3$) was hydrolysed by *Candida rugosa* lipase in 0.05 *M* acetate buffer at pH 4.5 at 30°C. Aliquots of the reaction mixture were extracted with *n*-hexane and analyzed by chiral HPLC. By following the decrease in the area for the ester peak the reaction was stopped at 50% conversion. The enantiomeric excess of the product (*R*)-cyanohydrin as determined by chiral HPLC was found to be 73.1% which is close to the value of 70.2% at 50.7% conversion reported by Mitsuda et al. [13] by HPLC analysis after derivatization to laurate.

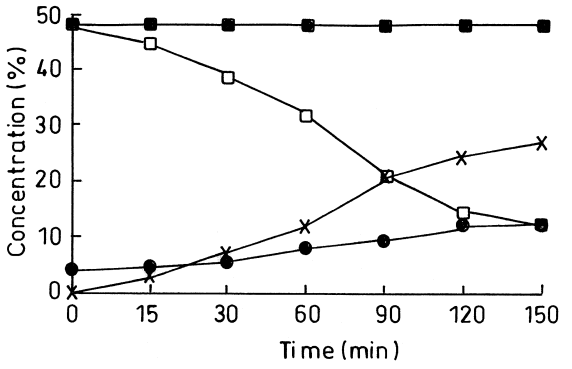


Fig. 3. Changes in concentrations of reactants and products observed by chiral HPLC during enantioselective alcoholysis of (*R,S*)-cyano(3-phenoxyphenyl)methyl butyrate with *n*-butanol catalyzed by *Pseudomonas cepacia* lipase immobilized on celite in *n*-hexane: □ (*S*)-ester, × (*S*)-cyanohydrin, ■ (*R*)-ester, ● 3-phenoxybenzaldehyde. Reaction was carried out with 0.17 *M* ester (100 ml, 5% solution) in *n*-hexane containing 1.26 g *n*-butanol and 1 g *Pseudomonas cepacia* lipase immobilized on 5 g celite at 30°C.

### *3.2. Calibration, reproducibility and limits of detection*

The aldehyde, the esters and the cyanohydrins were calibrated from 20 m*M* down to 1 m*M* by injecting 20 μl of the stock solution to the injector. The regression coefficients were in the range 0.998–0.999 for each ($n=10$). When a mixture of equimolar solutions of the aldehyde, the butyrate ester, the acetate ester and the cyanohydrins was injected, it was found that the relative response factor for the butyrate ester (sum of *R* and *S*), the acetate ester and the cyanohydrins were found to be the same while that for the aldehyde it was 3.94 compared to the ester. The reproducibility of retention times was found to be ±5% and for the peak area between ±0.6%. After 20 injections, the retention times shift by 0.5 to 0.7 min but after thorough washing of the column, switch back to original values. The calculated limit of detection for the esters and the cyanohydrins is 20 m*M* (maximum) and 2 m*M* (minimum) for reliable e.e. values. The optimum concentration to be used for analysis is 5 m*M*. We have obtained reproducible results with more than 20 batches of alcoholysis reaction with *Pseudomonas cepacia* lipase and after more than 100 injections.

The enantiomeric excess for the cyanohydrins as determined by chiral HPLC was compared with that obtained by mixing the optically pure (*S*)-cyanohydrin (e.e.>99%) with the (*R*)-cyanohydrin (e.e. 73.1%) in various proportions (from 1: 1 to 1:5). The enantiomeric excess values expected on the basis of optical rotations were reasonably close but it should be pointed out that purification of the cyanohydrins and the esters by column chromatography was a long and tedious process, and although the presence of 3-phenoxybenzaldehyde in the $^1$H-NMR spectra was not detected, its presence could be detected in the HPLC analysis due to its strong chromophore. Another important factor to be noted is that the sign of rotation for the ester and cyanohydrin of opposite configurations is same and contamination of the product cyanohydrin with unreacted ester would give misleading results. The chiral HPLC method presented here is far more reliable in determining the enantiomeric excess of the cyanohydrins.

## 4. Conclusion

A simple and reliable procedure for determination of enantiomeric excess of industrially important intermediate (*S*)-α-hydroxy-3-phenoxybenzeneacetonitrile **5**, and its *n*-butyrate ester by chiral HPLC is presented. The technique does not need any derivatization and can be used for routine analysis during the manufacturing process using either the acetate ester or the butyrate ester. Since the peaks are well resolved, the analytical procedure is also useful in the case of the manufacture process employing HCN addition to 3-phenoxybenzaldehyde.

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