# Short Communication

# Determination of the enantiomeric composition of samples of cocaine by normal-phase high-performance liquid chromatography with UV detection

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ABSTRACT

A high-performance liquid chromatographic method has been developed for the quantitation of the enantiomers of coca tures of the naturally occurring (−)-cocaine and synthetically produced (+)-cocaine were hydrolyzed in water to (+) and (− ecgonine. Esterification with an optically pure 2-octanol resulted in diastereoisomers that could be separated on bare silica ge acetonitrile–aqueous ammonium phosphate mobile phase.

INTRODUCTION

(−)-Cocaine is the naturally occurring alkaloid produced by the coca plant (erythroxylon coca). In 1923 a method for the synthesis of (±)-cocaine was published [1]. The racemic mixture was resolved into the pure (+) and (−) isomers by crystallizations with tartaric acid. Modifications [2,3] of this method and a new synthetic sequence [4] have been reported.

Since recent studies [5–8] indicate that the pharmacology and metabolism of the (+)-isomer may be very different from the natural (−)-cocaine, it would be useful to have a convenient method for the quantitative determination of the enantiomeric composition of small samples of cocaine. A high-performance liquid chromatographic (HPLC) separation of (±)-cocaine on two 25-cm β-cyclodextrin columns in series requiring a 90-min elution has been reported [9].

Eskes [10] developed a thin-layer chr graphic (TLC) method for the detection enantiomer of cocaine. Samples of cocaine v drolyzed with 2 *M* hydrochloric acid to e which was then esterified with (+)- or (−) nol by the action of benzenesulfonyl chlorid ridine [11]. The resulting diastereomers c separated on silica gel TLC.

It was felt that a similar procedure which only the methyl ester and left the benzoyl tact would allow convenient detection witl monitor at 254 nm. Since it is known that cc hydrolyzed by neutral water to benzoyl e [12], this seemed feasible. As the use of ba gel with aqueous eluents for the separation lipophilic amines is well known [13,14], we gated the use of such a system.

## MATERIALS AND METHODS

### Instrumentation

HPLC was performed with a Perkin-Elmer (Norwalk, CT, USA) series 3B pump, a Rheodyne (Cotati, CA, USA) 7125 injection valve fitted with a 2.0-ml loop, a Phenomenex (Rancho Palos Verdes, CA, USA) Spherisorb 5 silica column (250 × 4.6 mm I.D.) and a Knauer (Dusseldorf, Germany) variable-wavelength UV detector operated at 254 nm. Integrations were performed by a Hewlett-Packard (Palo Alto, CA, USA) 3390A integrator at an attenuation of 1 or 2. The solvent system was acetonitrile–0.001 $M$ ammonium phosphate, dibasic (pH 7.4) (75:25) with a flow-rate of 2 ml/min.

### Materials

(−)-Benzoyl ecgonine (BE), and (+)- and (−)-cocaine (COC) were kindly provided by Research Triangle Institute (Research Triangle Park, NC, USA). Benzenesulfonyl chloride and S-(+)- and R-(−)-2-octanol (99% optical purity) were obtained from Aldrich (Milwaukee, WI, USA). Pyridine was distilled from BaO and stored over KOH pellets. Reactions were carried out in 1-ml V-vials with PTFE-faced screwcaps (Wheaton, Millville, NJ, USA).

### Procedure

COC (0.5–1 mg; 0.0015–0.003 mmol) and 200 $\mu$l of water in a tightly capped reaction vial were heated in an oil bath at 130°C for 20 to 30 min. The cap was removed and heating continued under a stream of nitrogen. When the water had evaporated, two 200-$\mu$l portions of acetonitrile were added and blown dry.

After the vial had cooled to room temperature, 50 $\mu$l of pyridine, 5 $\mu$l (0.032 mmol) of the appropriate 2-octanol and 3 $\mu$l (0.023 mmol) of benzenesulfonyl chloride were added. After 15 min at ambient temperature, the pyridine was evaporated under a stream of nitrogen with gentle warming. To the residue were added 200 $\mu$l of acetonitrile and 1 $\mu$l of this solution was chromatographed. In the event of incomplete esterification, the acetonitrile was evaporated and the derivatization procedure repeated with the residue.

When the progress of the esterification was monitored, 5-$\mu$l aliquots of the pyridine solution were removed and diluted with 200 $\mu$l of acetonitrile. These solutions were evaporated and the residue redissolved in 100 $\mu$l of acetonitrile and chromatographed.

To analyze mixtures of (+)- and (−)-COC of known composition, standard solutions of each were prepared in acetonitrile and appropriate volumes of each were added to the reaction vial and blown to dryness before the hydrolysis step.

The relative rates of reaction of the enantiomers of BE with the enantiomers of 2-octanol were determined by reacting each pure COC isomer with an excess of racemic alcohol. The relative amounts of the two diastereomeric esters formed is proportional to relative rates of reaction of each of the alcohols with the isomerically pure COC.

## RESULTS AND DISCUSSION

Under the conditions described 75–85% of the COC was hydrolyzed to BE. As this step is not enantioselective, there was no need to take the hydrolysis to completion. If heating was continued much after the evaporation of water, decomposition or sublimation resulted in a significant loss of mass.

The esterification of BE using the method of Eskes [9] was rapid and quantitative. In cases where the reaction was incomplete, it was probably the result of the presence of moisture rather than insufficient reaction time. As would be expected, the enantiomers of BE reacted with the enantiomers of 2-octanol at different rates. The esterification of (−)-BE with (−)-2-octanol was 1.4 times as fast as with (+)-2-octanol. With (+)-BE the ratio of (+/+) to (+/−) was also 1.4. Thus for an accurate determination of the enantiomeric composition of the BE produced by the hydrolysis of COC samples, it is necessary of react all of the BE with enantiomerically pure alcohol, otherwise the faster reacting enantiomer will appear in erroneously high proportion.

The diastereometric esters from BE and 2-octanol had nearly baseline separation with the (+)-BE-(−)-2-octyl ester (+/−), or its enantiomer (−/+), eluting 1 min before (+)-BE-(+)-2-octyl ester (+/+) or its enantiomer (−/−) (Fig. 1 shows the relevant portion of the chromatogram). The resolution (the distance between the peak centers divided by
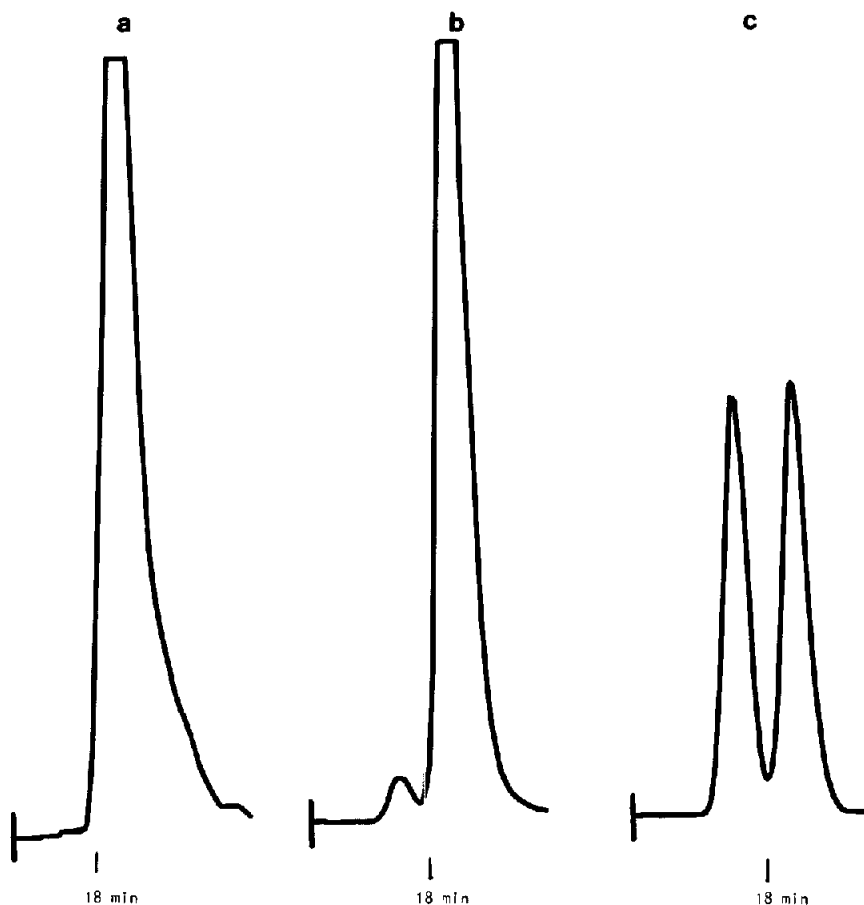
Fig. 1. (−)-2-Octyl esters from reaction with benzoyl ecgonine mixtures resulting from the hydrolysis of (a) (−)-COC, (b) 95% (−)-COC, 5% (+)-COC and (c) 50% (−)-COC, 50% (+)-COC.

the average peak width) of the diastereometric esters was 1.1 and the separation factor was 1.06. The relationships between the actual composition of mixtures of (+)- and (−)-COC and the integrated peak areas of their 2-octyl ester derivatives are given in Fig. 2. As can be seen from Fig. 2, a calibration curve should be obtained in each case as the integrator processes the leading peak (○) slightly differently than it does the trailing peak (●). Even without taking this systematic variation into account the correlation coefficient for the data in Fig. 2 was 0.997. Multiple injections of a solution showed a standard deviation of 1%.

Fig. 3 shows a typical chromatogram with retention times.

The commercial availability of both $S$-(+)- and $R$-(−)-2-octanol allows the enantiomeric analysis to be tailored to cause the minor component to elute first for greater sensitivity. Analyzing for contamination by (−)-COC in a preparation of (+)-COC by derivatization with (+)-2-octanol results in the elution of the minor component before that of the major one, making it easier to more accurately determine optical purities when small amounts of the minor isomer are present. While no attempt was made to optimize the sensitivity of this assay, it was capable of quantitating a 5% enantiomeric contamination in a 500 $\mu$g sample of cocaine.
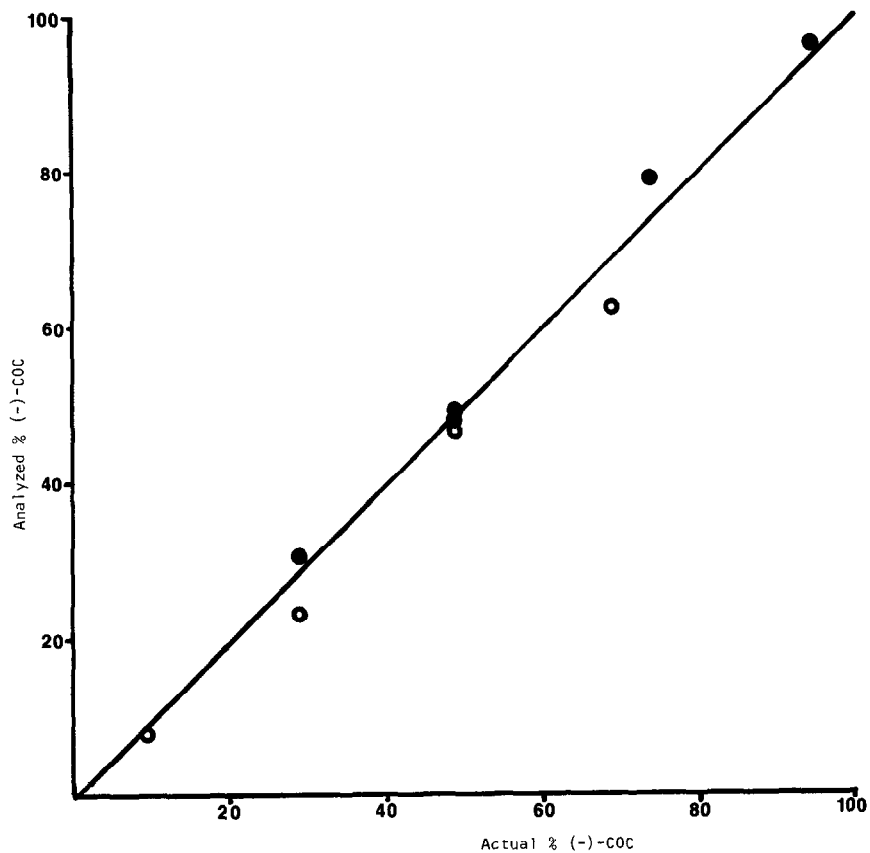
Fig. 2. Comparison of the analysis of enantiomeric purity of ( − )-COC versus the actual composition of ( ± ) mixtures. Reaction with (●) ( − )-2-octanol and (○) ( + )-2-octanol.
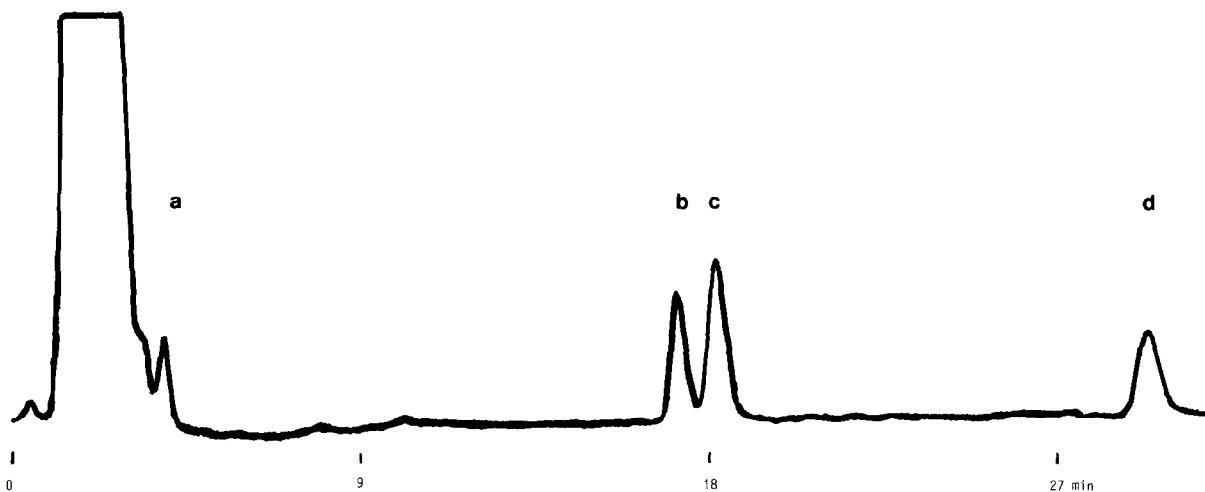


Fig. 3. Chromatogram of the reaction mixture of benzoyl ecgonine, from the hydrolysis of ( − )-cocaine, and racemic 2-octanol after 5 min reaction. Peaks: a = ( − )-Benzoyl ecgonine (3.8 min); b = ( − )-benzoyl ecgonine-( + )-2-octyl ester (17.6 min); c = ( − )-benzoyl ecgonine-( − )-2-octyl ester (18.6 min); d = ( − )-cocaine (30.2 min).

## ACKNOWLEDGEMENTS

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