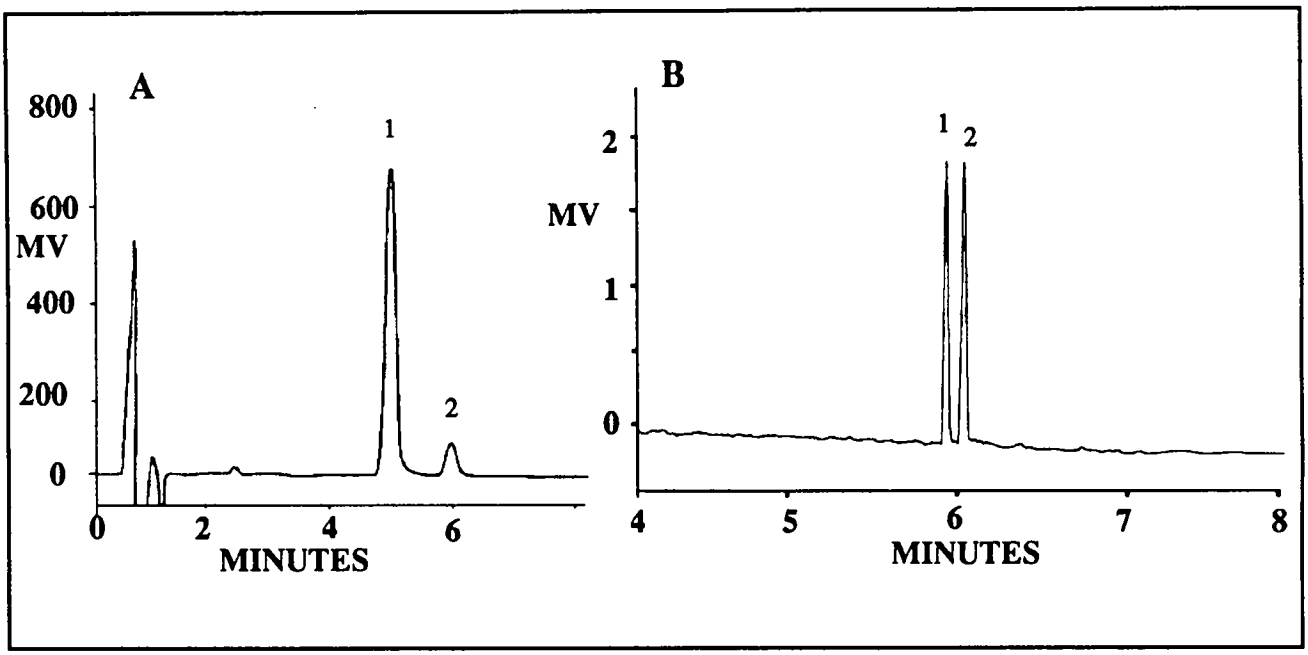
PHARMACEUTICAL Application Briefs

930793

Rx Prescription for success

Rx 021 8/90

HPLC PURIFICATION OF INTERMEDIATE SYNTHESIS PRODUCT FOLLOWED BY CAPILLARY ELECTROPHORESIS ENANTIOMER SEPARATION



CONDITIONS ON WATERS QUANTA™ 4000

MODE:	FZCE
BUFFER:	50 mM Sodium Phosphate Buffer pH = 7 Saturated with ß-Cyclodextrin
MODIFIER:	20 % MEOH
CAPILLARY:	60 cm x 75 µm i.d.
VOLTAGE:	+ 20 KV
DETECTOR:	UV @ 214 nm
INJECTION:	5 sec x 10 cm Hydrostatic

SAMPLE MATRIX:

A. Unpurified Synthesis Reaction Mixture

B. Purified Fraction from HPLC approximately 0.1mg/ml

PEAK IDENTIFICATION:

A. HPLC Purification
1. Desired Product
2. Impurity

B. CE Enantiomer Separation
1. Intermediate Synthesis Product (-)
2. Intermediate Synthesis Product (+)

REFERENCE:
John Van Antwerp and Larry Mugavero, Application Chemists, Morristown, N.J. Laboratory

Author: Peter Rahn

INTERESTING FACTS

1. Capillary electrophoresis represents a new technique available for chemists to achieve chiral separations with minimal sample preparation. This separation is achieved by the judicious choice of buffers, pH, organic modifier and the chiral discriminator- ß-cyclodextrin.

2. The initial crude synthesis product was purified by reverse phase HPLC using a Nova-Pak C-18 3.9 mm x 15 cm column. Capillary electrophoresis was performed on the purified product to verify the enantiomer ratio.

3. This CE separation generated over 1,400,000 plates (one million four hundred thousand) with a total analysis time of only 6 minutes. This illustrates the very high efficiency available to achieve chiral separations by CE. Even if the enantiomer ratio was 99:1, these enantiomers are well resolved for good quantitation.

4. A similar separation by HPLC using chiral columns would require 20 - 40 minutes. CE eliminates both specialized stationary phases and derivatization to achieve this separation.

5. The excellent signal to baseline noise level noted in this electropherogram is typical of the Quanta 4000's performance using the discretely variable UV/VIS detector. The data was collected on the 860 VAX based system.

6. Other chiral separations performed on the Quanta 4000 are presented in Rx 017 8/90 through Rx 022 8/90.

7. Electrolyte is prepared by saturating a 50 mM sodium phosphate pH = 7 buffer with ß-cyclodextrin. The solution is filtered and 20% methanol is added as an organic modifier.