Derivatization and High Sensitivity HPLC and MECC Analysis of Ephedrine and Pseudoephedrine Using a Fluorescent Reagent, 6-Aminoquinolyl-N-Hydroxysuccinimidyl Carbamate

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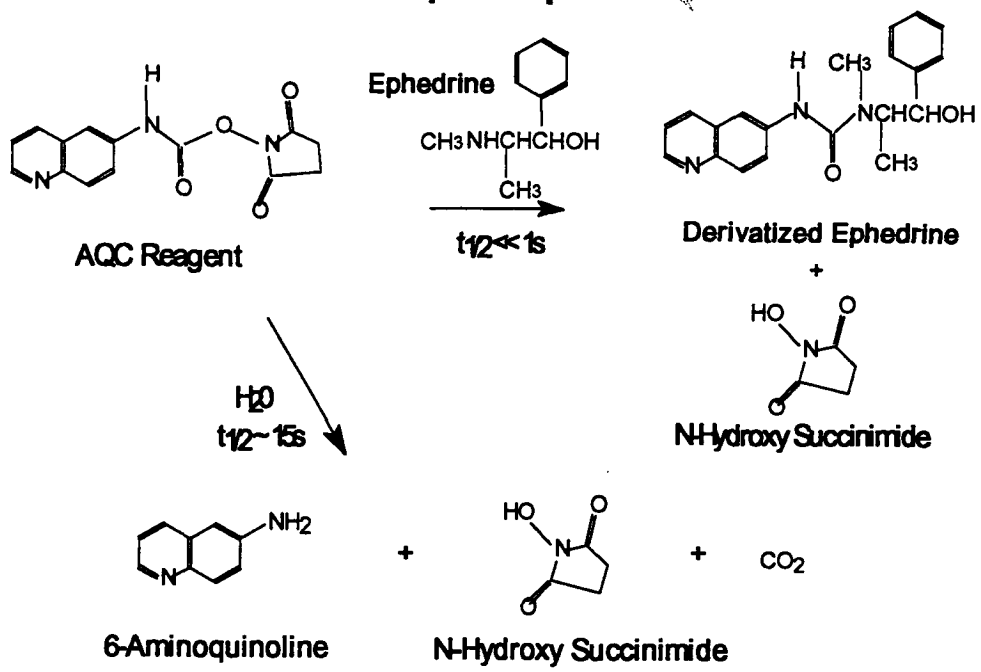
Abstract

Derivatization methods in analytical chemistry are an important tool for the analysis of reactive species, particularly amines, which may lack easily detected functionalities. We have recently developed a new reagent, 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC, Waters AccQ·Fluor reagent), that reacts with both primary and secondary amines and brings simplicity to the derivatization procedure while providing superb linearity, reproducibility and accuracy for amino acid [1]. This work will describe the analysis of the amine drugs ephedrine and pseudephedrine which have now also proven amenable to derivatization by AQC. This method retains many of the same advantages previously demonstrated for amino acids. For example, the analysis sensitivity is significantly enhanced by the labeling procedure, with detection limits in the femtomole range. In addition, the selectivity afforded by the fluorescent tag is often highly favorable for analytes in a biological matrix, and as a consequence, samples often require little or no sample cleanup. Derivatization is simply carried out by buffering the sample and adding reagent. Removal of the hydrolysis byproduct is not required, as its fluorescence emission maximum is shifted over 100nm from the maxima of the derivatized analytes. Typical methods for trace analysis of amine drugs in plasma use solvent extraction with ethyl acetate followed by derivatization of the buffered extract. The samples are then injected with no further cleanup. These procedures have enabled the analysis of these analytes in plasma at trace levels (detection limits <0.1mM). The achiral derivatization also serves to enhance selectivity for separation of the drug enantiomers. Separations by either HPLC on B-cyclodextrin bonded columns or by capillary electrophoresis using optically active surfactants as electrolyte additives are feasible. Thus, with a single preparation, the same sample can be used for both high sensitivity achiral analysis and highly selective enantiomeric analysis of a drug analyte.

[1] S. A. Cohen and D. P. Michaud, Anal. Biochem, 211 (1993) 279-287.

Chemistry of Derivatization with AQC

Example: Ephedrine



Analysis of Drugs in Plasma

Chromatography

Column: Symmetry C18, 3.9 x 150 mm
Mobile Phase A: 70 mM NaOac, 8.5 mM TEA, pH 5.05
Mobile Phase B: Acetonitrile
Flow Rate: 1 ml/min
Column Temperature: 42C (pseudoephedrine)
30C (ephedrine)
Gradient:
pseudoephedrine: 20%-30% B (2 min),
30%-40% B (10 min)
ephedrine: 20%-30% B (2 min),
30%-35% B (10 min)

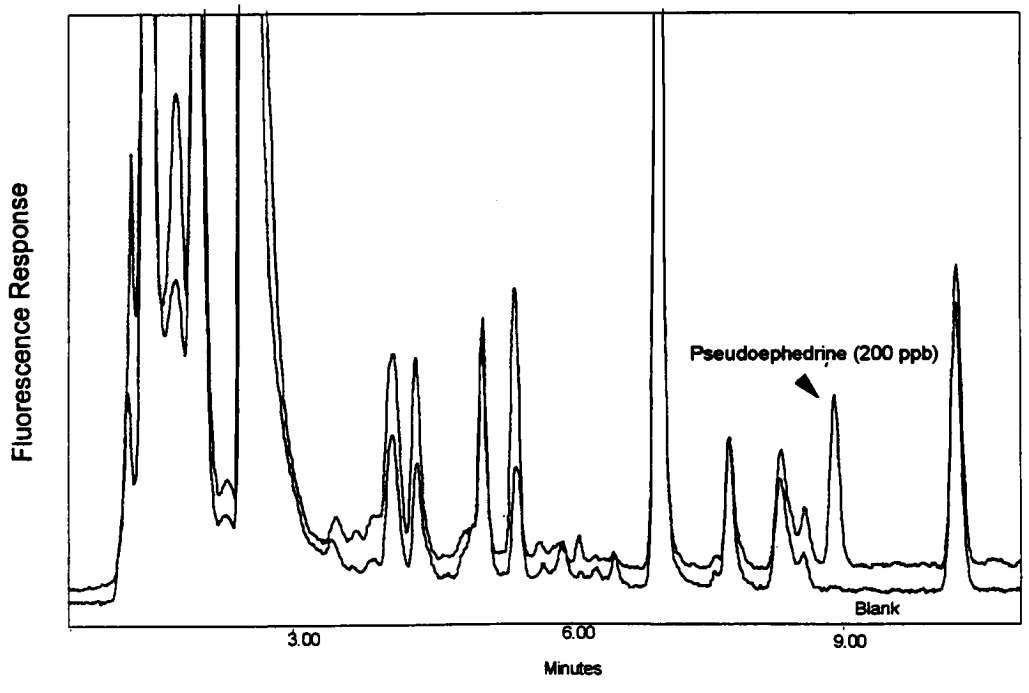
Analysis of Drugs in Plasma

Sample Preparation

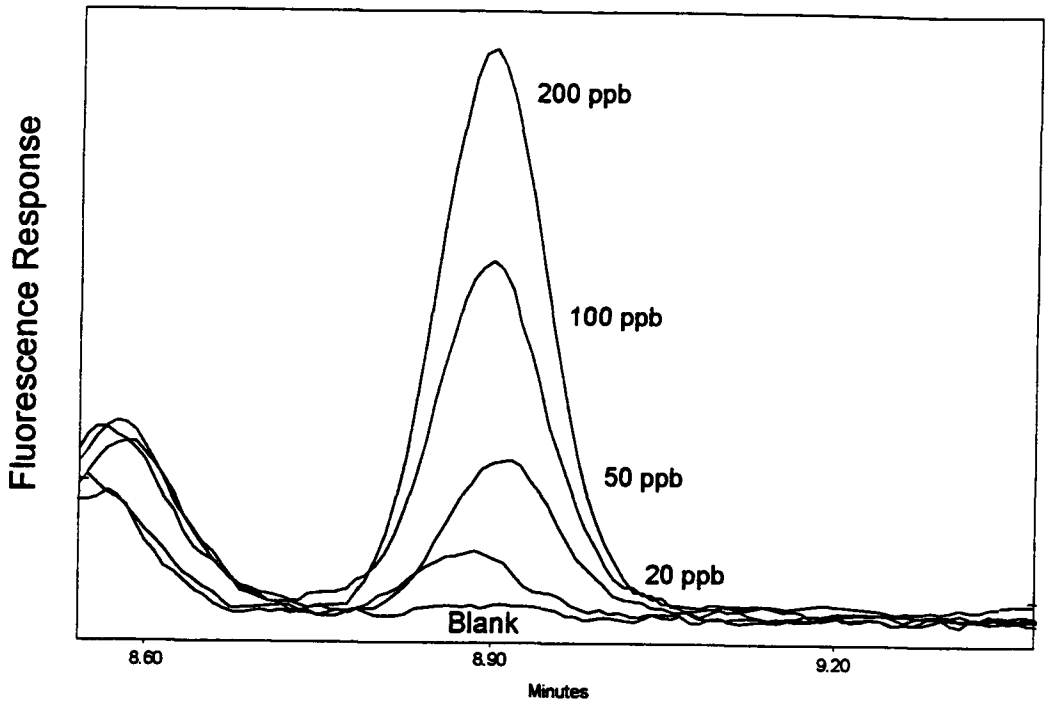
1. Add 20 µl 6N NAOH to 100 ul plasma
2. Add 1 ml EtOAc, vortex
3. Centrifuge 5 min at 4000 RPM
4. Dry 800 µl supernatant
5. Reconstitute in 100 ul borate buffer, pH 8.8
6. Add 40 µl AQC solution
7. Inject 50 µl

Pseudoephedrine Analysis

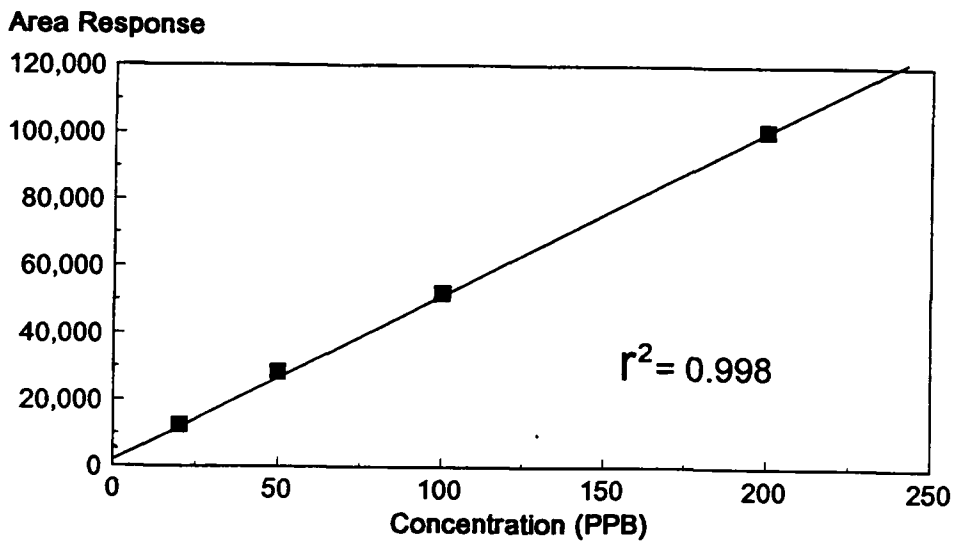
Overlay of Plasma and 200 ppb Sample



Analysis of Pseudoephedrine in Plasma

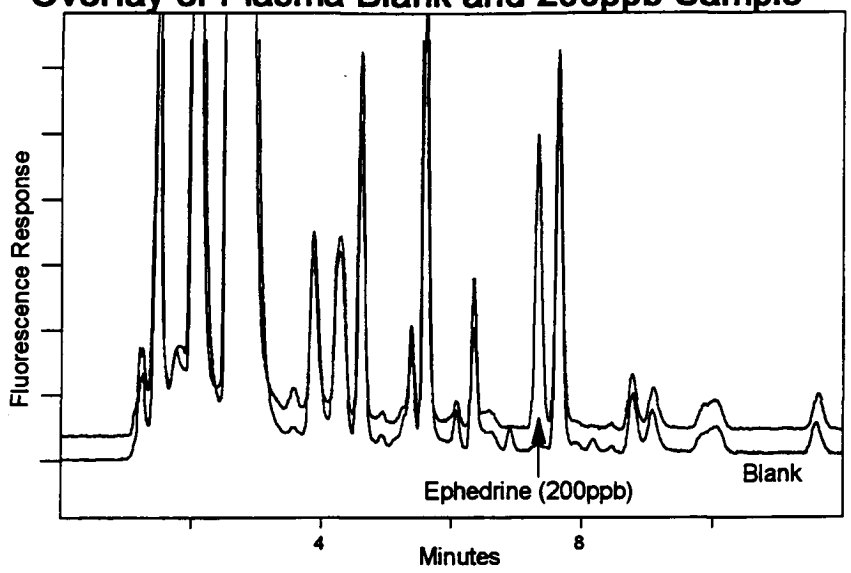


Response Linearity for Pseudoephedrine

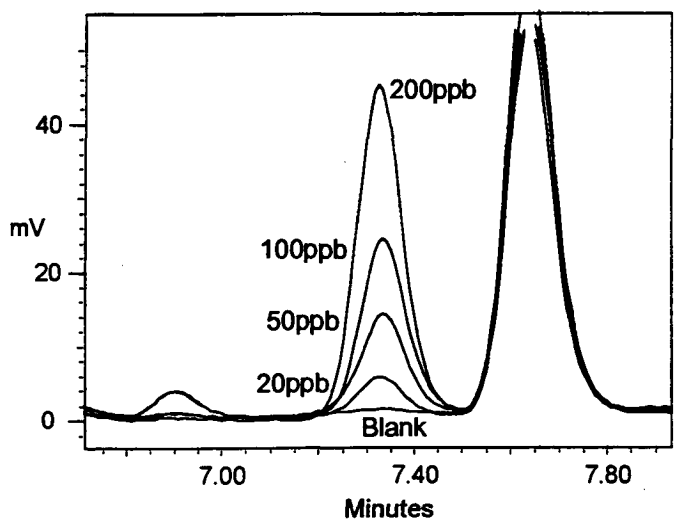


Ephedrine Analysis

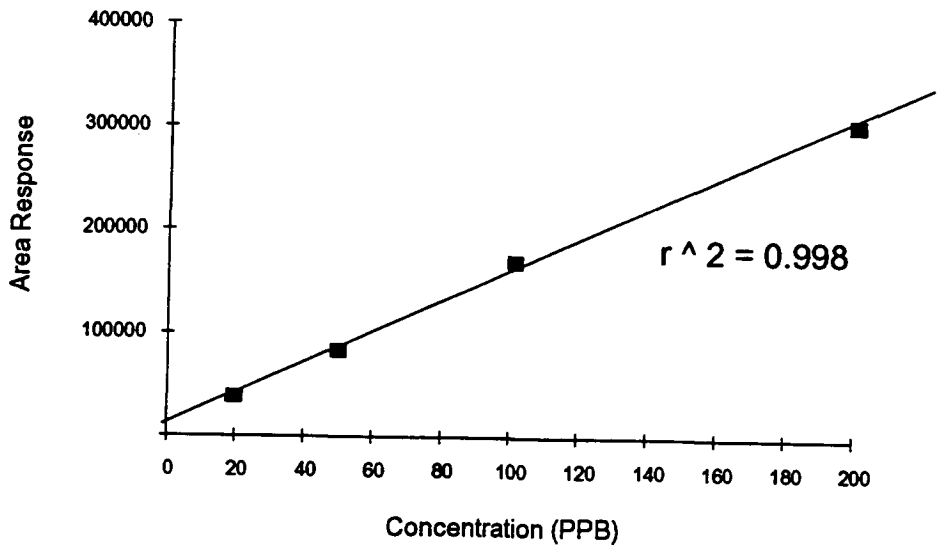
Overlay of Plasma Blank and 200ppb Sample



Analysis of Ephedrine in Plasma

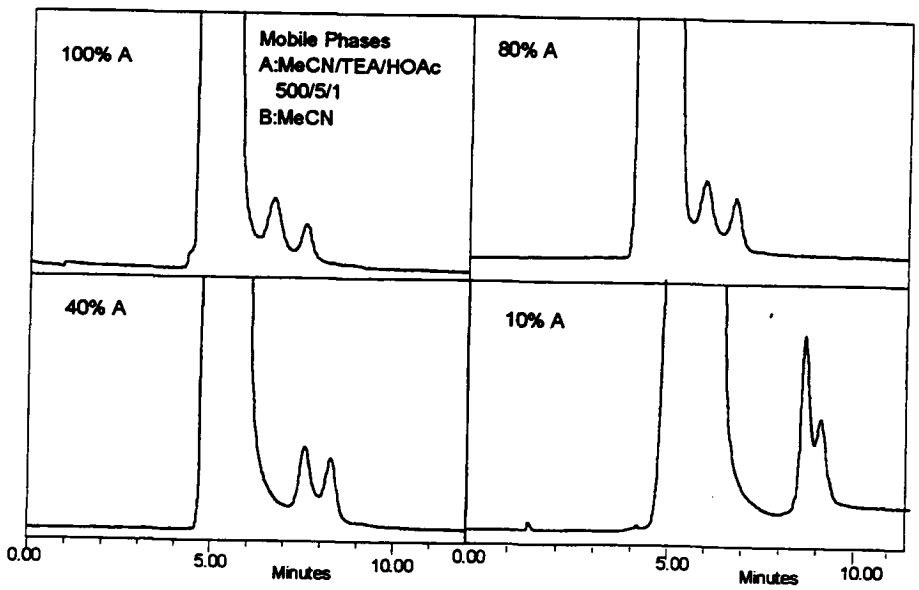


Response Linearity for Ephedrine

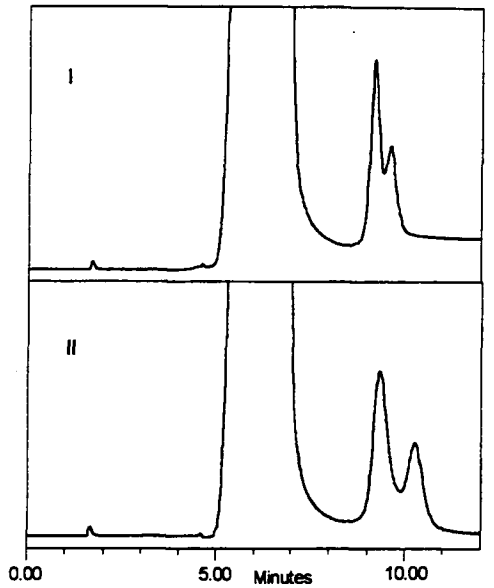


Optimization of Chiral HPLC SEPARATION AQC-Ephedrine Analysis

Column: β-cyclodextrin (Cyclobond I 2000)



Optimization of Chiral HPLC Separation
AQC-Ephedrine Analysis
Column: β-cyclodextrin (Cyclobond I 2000)



Mobile phase
I. A: MeCN/TEA/HOAc
500/5/1
B: MeCN

10% A

II. A: MeCN/TEA/HOAc
500/6/1
B: MeCN

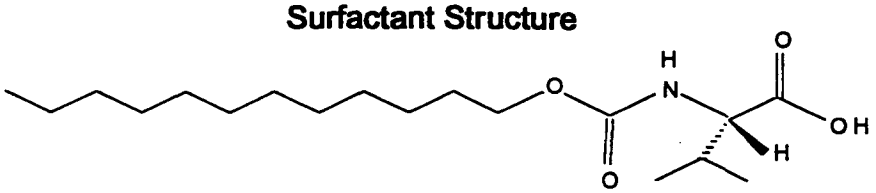
10% A

Effect of changing base/acid ratio

CE Experimental Conditions

CE System:	Q4000E (Waters)
Capillary:	50 micron by 60 cm
Detection:	UV @ 254 nm
Data:	5 pts/sec
Injection:	10 Sec. Hydrostatic
Voltage:	+15 KV
Electrolyte:	25mMPO4/BO4, pH9.1
	15-75mM Surfactant
Sample:	AQC-Ephedrine and -Pseudoephedrine

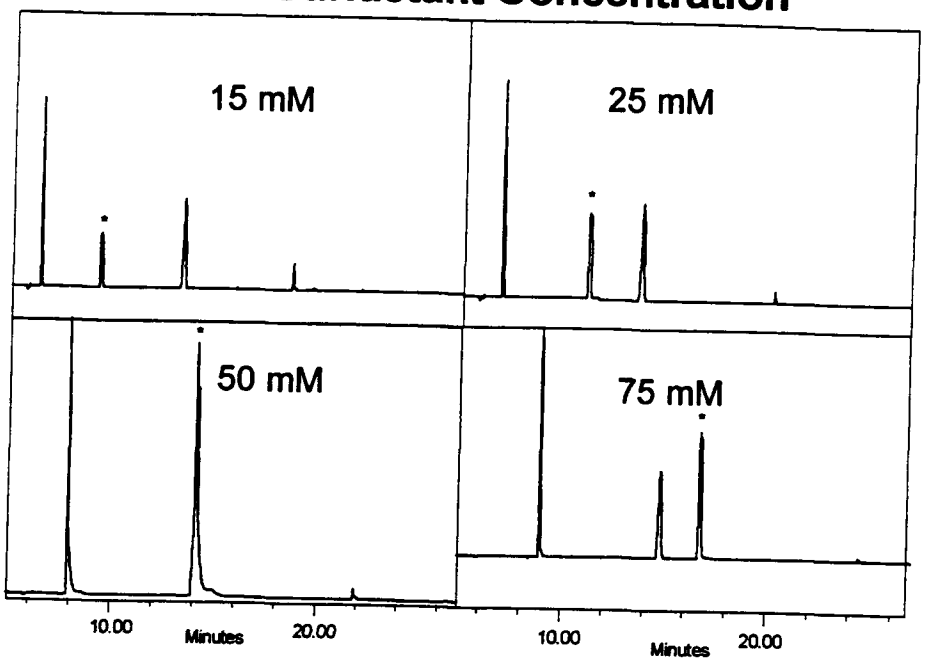
Surfactant Structure



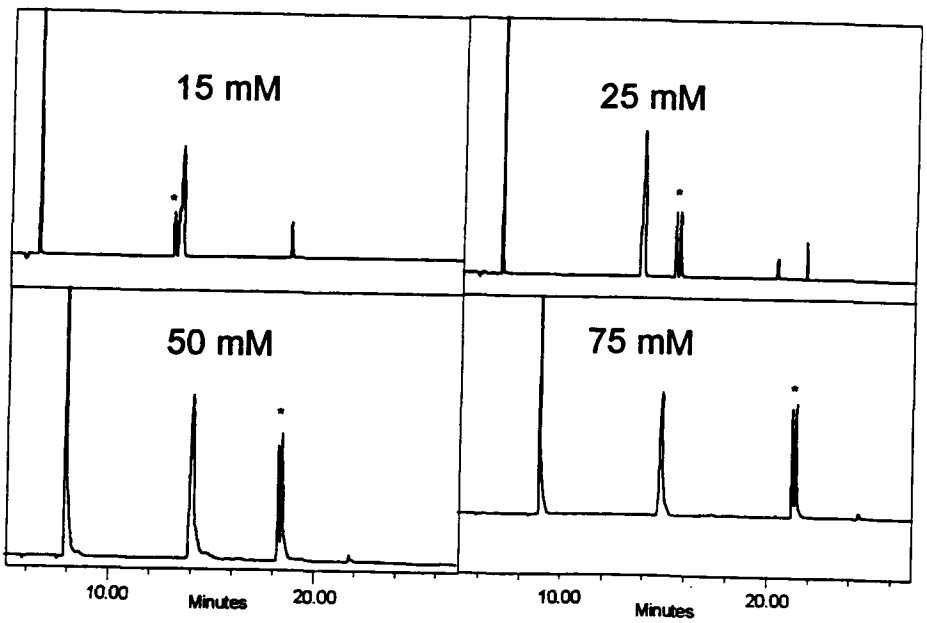
(S)-N-dodecoxycarbonylvaline

MECC Separation of AQC-Ephedrine

Effect of Surfactant Concentration



MECC Separation of AQC-Pseudoephedrine

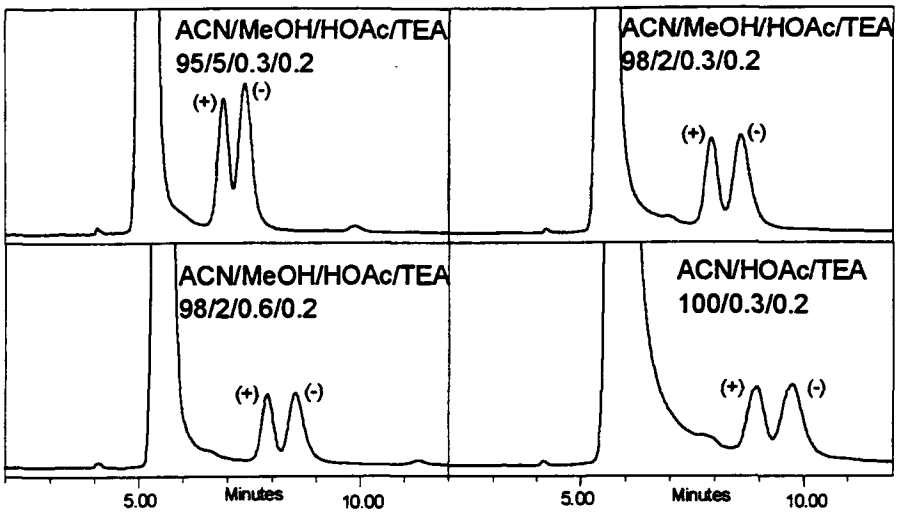


Resolution of enantiomers in MECC of neutral compounds is optimized by varying surfactant concentration.

Optimization of Chiral HPLC Separation

AQC- Pseudoephedrine Analysis

Column: β-cyclodextrin, S-naphthylethyl(Cyclobond I 2000 SN)



Resolution is optimized by varying the ratio acid:base; retention is optimized by the addition small amounts of MeOH.The AQC tag provides improved selectivity as well as greatly increasing detection sensitivity.

Summary of Drug Derivatization

- AQC is an effective and versatile derivatization reagent which can be used for high sensitivity drug analysis
- Reactions are quantitative
- Linear response
- Little or no sample prep required
- Detection is highly sensitive
- In Chiral HPLC separation as well in MECC AQC reagent provides improved selectivity and increased detection sensitivity