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**Analysis of Pharmaceuticals By
Capillary Electrophoresis
With Indirect UV Detection**

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Abstract

Indirect ultraviolet (UV) detection has been used in liquid chromatography (LC) for a number of years. In practice, a UV absorber is added to the mobile phase to produce a high background absorbance. As each analyte of interest elutes from the column, a displacement occurs, allowing more light to reach the photodiode sensor in the detector. This increase in transmittance generates a signal that is directly proportional to the amount of each analyte present. This technique is somewhat limited in LC however, due to system peak problems stemming from interactions of the UV absorbing molecule with the chromatographic stationary phase. This is not the case with capillary electrophoresis (CE). These system peaks are greatly diminished in CE owing to considerably decreased or non-existent interaction of the UV absorbing molecules in the electrolyte with the capillary. The result is that CE analyses with indirect UV photometry can play an important role by offering the possibility of choice from a much larger number of molecules giving a high UV background. This allows for enhanced sensitivity of detection of many molecules that have poor chromophoric characteristics. This presentation will illustrate that by selecting the proper UV absorbing background electrolyte, optimum sensitivity, resolution, run time and peak symmetry can be achieved. It will also illustrate how CE with indirect UV detection extends the range of pharmaceutical applications to include the analysis of various compound classes.

Introduction

Capillary Ion Analysis (CIA) is a capillary electrophoretic (CE) technique optimized for the rapid analysis of highly mobile small molecular weight ions.

- Selectivity is based upon the mobility of the analyte in the electrolyte.
- Speed of Analysis is optimized by ensuring the EOF direction is always towards the detector. EOF is controlled by either pH or special electrolyte modifiers.
- Sensitive Detection is based predominately on the indirect UV principle, since the majority of the inorganic species lack chromophores

Ions Characterized by CIA

Anions Inorganic

arsenate
arsenite
azide
borate
bromate
bromide
carbonate
chlorate
chloride
chlorite
chromate
cyanide
fluoroborate
fluoride
hypochlorite
iodide
metasilicate
metavanadate
molybdate
monofluorophosphate
nitrate
nitrite
orthovanadate
perchlorate
persulfate
phosphate
phosphite
selenate
selenite
sulfate
sulfide
sulfite
thiocyanate
thiosulfate
tungstate

Anions Organic

acetate
trans-aconitate
ascorbate
dl-aspartate
benzoate
butanesulfonate
butyrate
4-carboxybenzaldehyde
chloroacetate
citrate
crotonate
decanesulfonate
dodecanesulfonate

Anions Organic

dichloroacetate
ethanesulfonate
formate
fumarate
galactarate
d-galacturonate
d-gluconate
glucuronate
l-glutamate
glutarate
glycerate
glycolate
glyphosate
heptanesulfonate
hexanesulfonate
α-hydroxybutyrate
hydroxymethylbenzoate
2-hydroxyvalerate
isocitrate
α-ketoglutarate
lactate
maleate
malonate
methanesulfonate
nonanesulfonate
octanesulfonate
orotate
oxalacetate
oxalate
pentanesulfonate
o-phthalate
propanesulfonate
propionate
pyridinedicarboxylate
pyruvate
quininate
salicylate
shikimate
sorbate
succinate
tartarate
terephthalate
trichloroacetate
trifluoroacetate
trimesate
p-toluate
valerate

Alkali Metals

lithium
sodium
potassium
rubidium
cesium

Alkaline Earths

beryllium
magnesium
calcium
strontium
barium

Transition Metals

manganese
iron
cobalt
nickel
copper
zinc
cadmium
mercury
lead

Lanthanides

lanthanum
cerium
praseodymium
neodymium
samarium
europium
gadolinium
terbium
dysprosium
holmium
erbium
thulium
ytterbium
lutetium

Non-Metal Cation

Ammonium

Peak Capacity

36 Anions in 83 Seconds (Analysis Time 2.9 Min.)

Electrolyte: 5 mM chromate

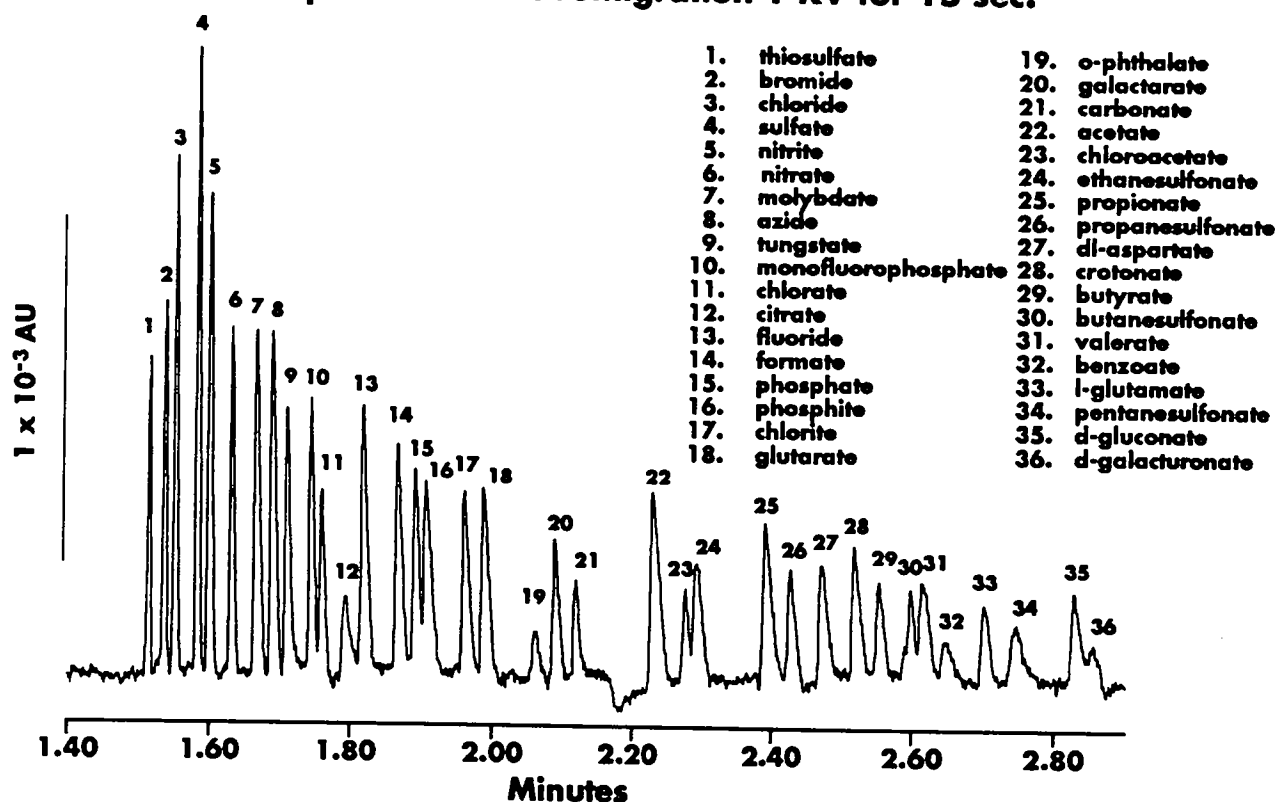
0.4 mM CIA-Pak™ OFM anion-BT, pH 8.0

Capillary: Waters AccuSep™ 50 μ m x 60 cm fused silica

Potential: 30 KV @ 18 μ A (negative)

Detection: 254 nm indirect

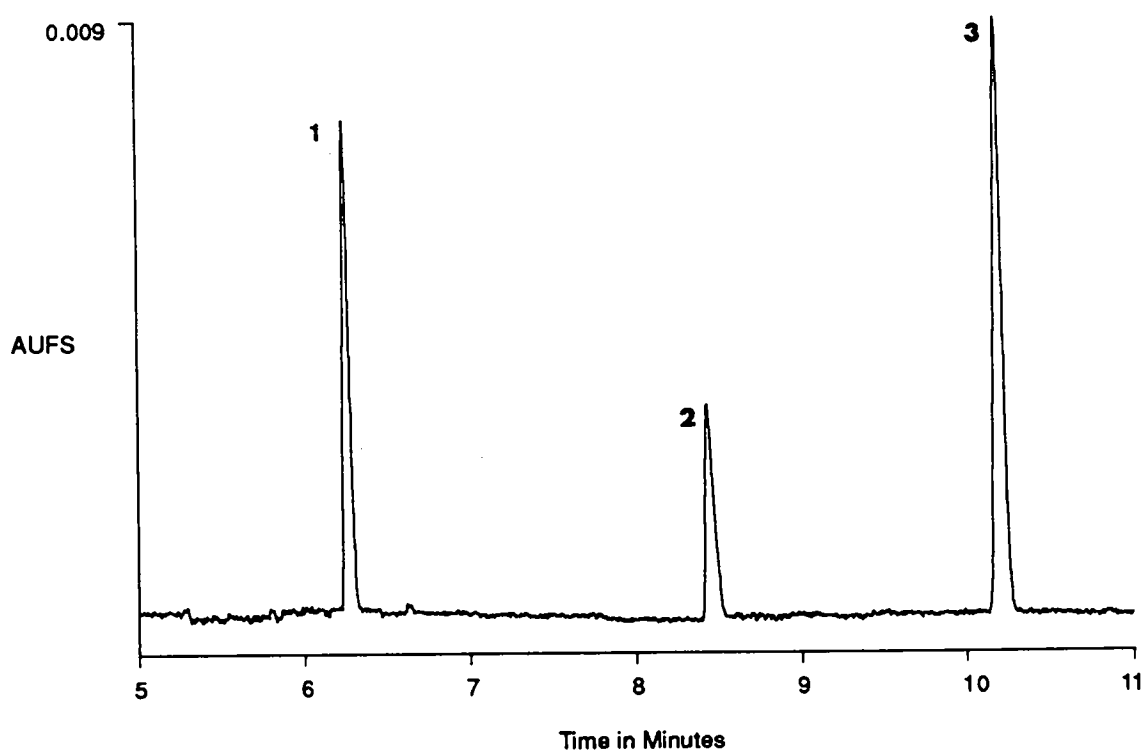
Injection: Electromigration 1 KV for 15 sec.



This is a recent anion separation illustrating the peak capacity of CIA methodology. The anions are in the 0.3 to 3.3 ppm concentration range. IC can do the same number of anions but requires 30 minutes and gradient elution.

Free Zone CE Separation of Organic Compounds

Free Zone Separation of Chlorpheniramine Maleate, Ephedrine Sulfate, and Propranolol Hydrochloride

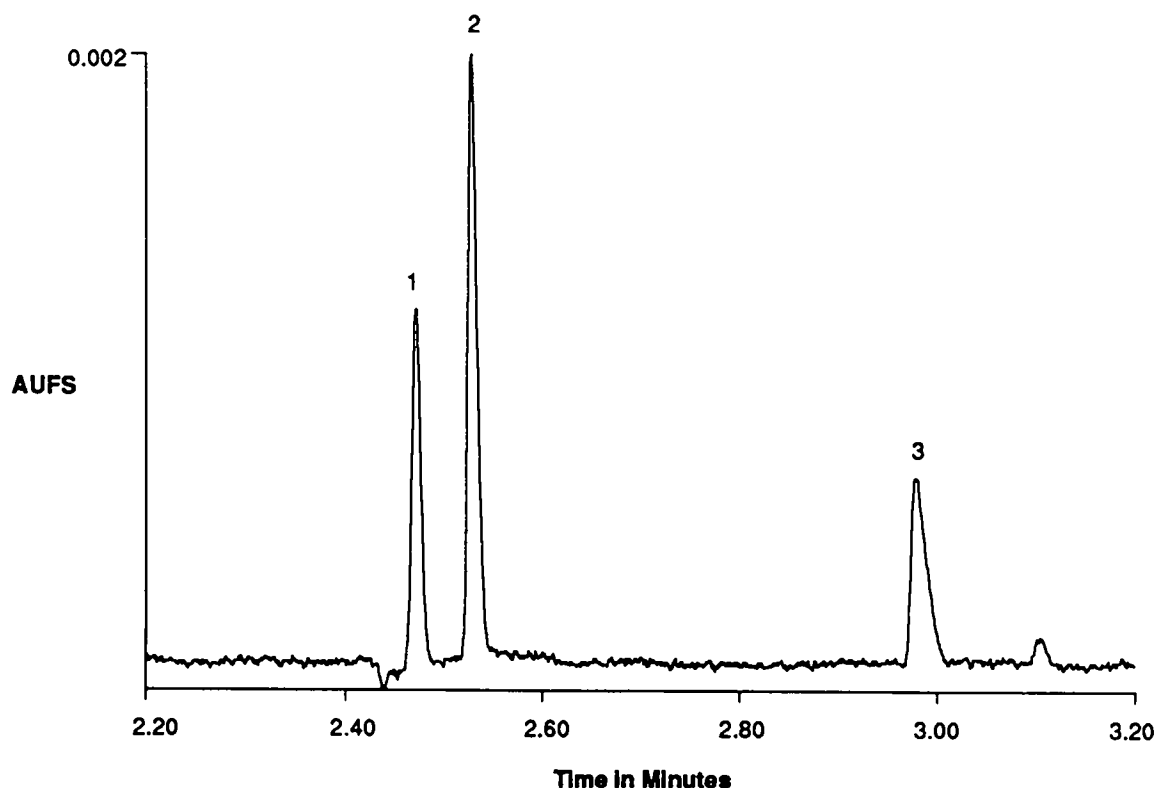


Quanta 4000 CE Conditions

Mode: Free Zone
Capillary: AccuSep 75 micron by 60 cm
Buffer: 25mM sodium phosphate, pH 2.5
Voltage: 30KV
Detection: UV @ 214 nm
Injection: 10 second Hydrostatic
Sample: 1) Chlorpheniramine Maleate, 25 PPM
2) Ephedrine Sulfate, 25 PPM
3) Propranolol Hydrochloride, 5 PPM

CIA Separation of Inorganic Salts Of Organic Bases

CIA Separation of Chlorpheniramine Maleate, Ephedrine Sulfate, and Propranolol Hydrochloride



Quanta 4000 Conditions

Mode: CIA
Capillary: AccuSep 75 micron by 60 cm
Electrolyte: 5 mM chromate, 0.4 mM
CIA-Pak OFM Anion-BT, pH 8.0
Run Voltage: 20KV (Negative)
Detection: UV @ 254 nm Indirect
Injection: 30 second Hydrostatic
Sample: 1) Chloride 3.75 PPM
2) Sulfate 3.75 PPM
3) Maleate 3.75 PPM

**Content Uniformity:
Free Zone and CIA Modes**

**Content Uniformity Results:
Ephedrine by Free Zone CE**

Sample	Avg. mg/mL	%Label
1	50.84	101.7
2	52.64	105.3
3	50.38	100.8
4	47.14	94.3
5	50.03	100.1
6	52.67	105.3
7	51.12	102.2
8	53.22	106.4
10	52.95	105.9

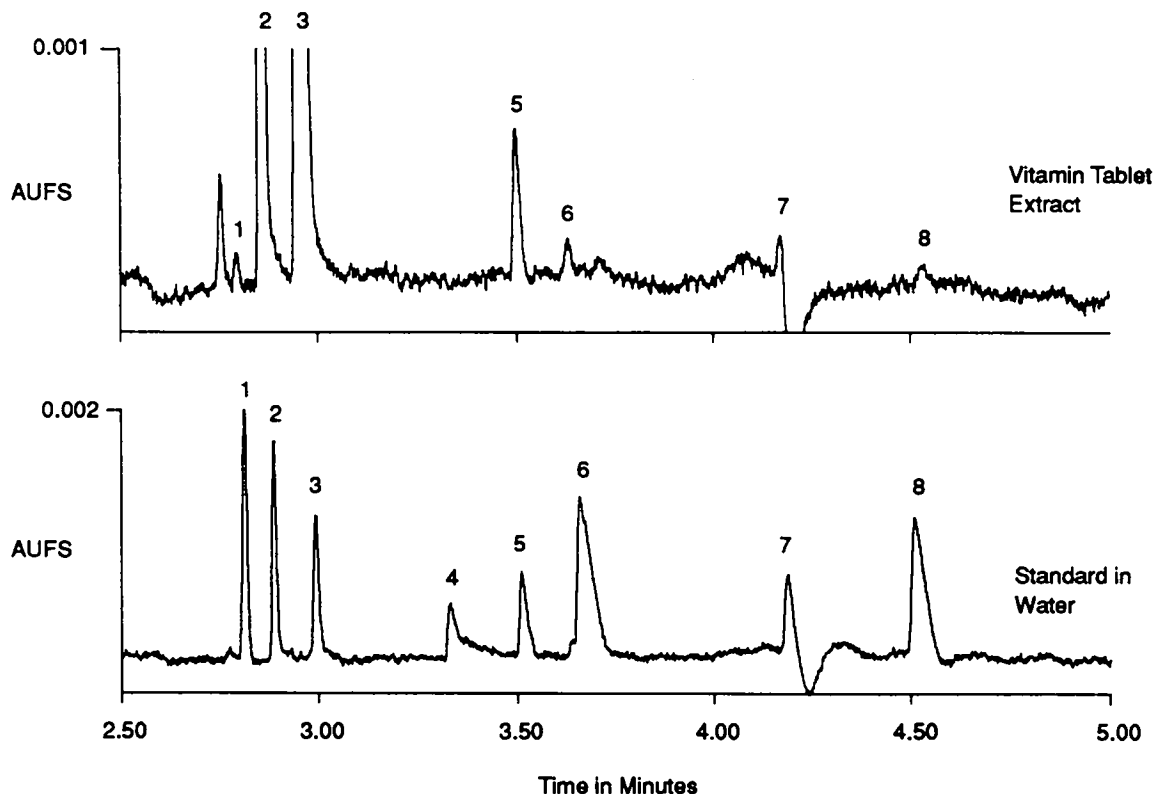
AVG 102.7
% RSD 3.6

**Content Uniformity Results:
Sulfate by CIA**

Sample	Avg. mg/mL	%Label
1	7.68	102.7
2	7.64	102.1
3	7.70	102.9
4	7.67	102.5
5	7.53	100.7
6	7.50	100.3
7	7.60	101.6
8	7.84	104.8
9	7.69	102.8
10	7.70	102.9

AVG 102.3
% RSD 1.2

CIA Separation of Anions: Vitamin Tablet Extract

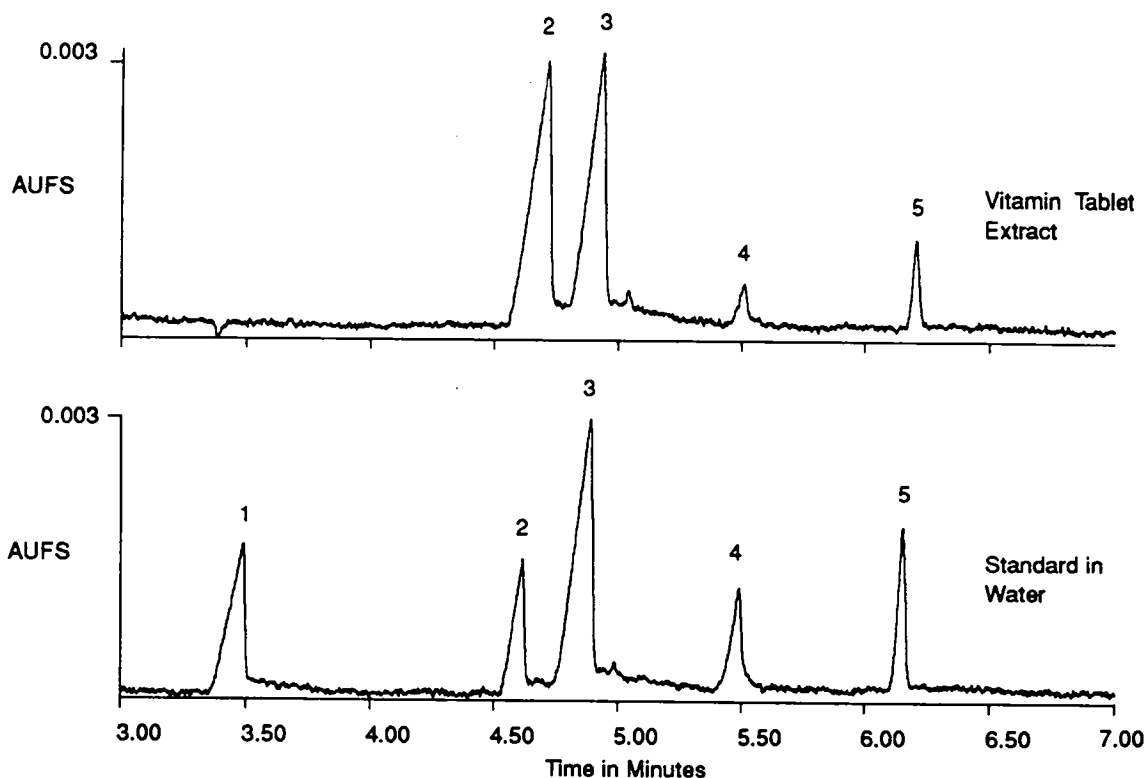


Quanta 4000 CE Conditions

Mode: CIA
 Capillary: AcuSep 75 micron by 60 cm
 Electrolyte: 5 mM chromate, 0.4 mM CIA-Pak OFM anion-BT, pH 8.0
 Voltage: 20KV (negative)
 Detection: UV @ 254 nm (indirect)
 Injection: 30 second Hydrostatic
 Peak ID STD: 1) Chloride 2 PPM 2) Sulfate 2 PPM
 3) Nitrate 2 PPM 4) Citrate 2 PPM
 5) Fumerate 2 PPM 6) Phosphate 2 PPM
 7) Carbonate Impurity 8) Acetate 2 PPM

Vitamin Tablet Sample Prep: 1) Add tablet to 500 mL water (pH 2.0 with Nitric acid).
 2) Filter and dilute 1/10 with water

CIA Separation of Cations: Vitamin Tablet Extract



Quanta 4000 CE Conditions

Mode: CIA
 Capillary: AcuSep 75 micron by 60 cm
 Electrolyte: 5 mM UVCat-1, 6.5 mM HIBA, pH 4.4
 Voltage: 20KV
 Detection: UV @ 185 nm (indirect)
 Injection: 30 second Hydrostatic
 Peak ID STD: 1) Potassium 1 PPM 2) Calcium 1 PPM
 3) Sodium 1 PPM 4) Iron 3PPM
 5) Zinc 1 PPM

Vitamin Tablet Sample Prep: 1) Add tablet to 500 mL water (pH 2.0 with Nitric acid).
 2) Filter and dilute 1/10 with water

CIA Reproducibility

Cation	%RSD Migr. Time	%RSD Area
Potassium	0.11	2.70
Calcium	0.12	3.20
Sodium	0.12	1.90
Iron	0.10	1.80
Zinc	0.10	2.40

Anion	%RSD Migr. Time	%RSD Area
Chloride	0.50	1.60
Sulfate	0.30	2.60
Nitrate	0.50	1.20
Phosphate	0.50	1.50

N = 5
1-4 PPM Levels

Conclusion

Wide Range of Applications

- Inorganic Anions
- Organic Anions
- Alkali Metals
- Alkaline Earths
- Transition Metals
- Lanthanides

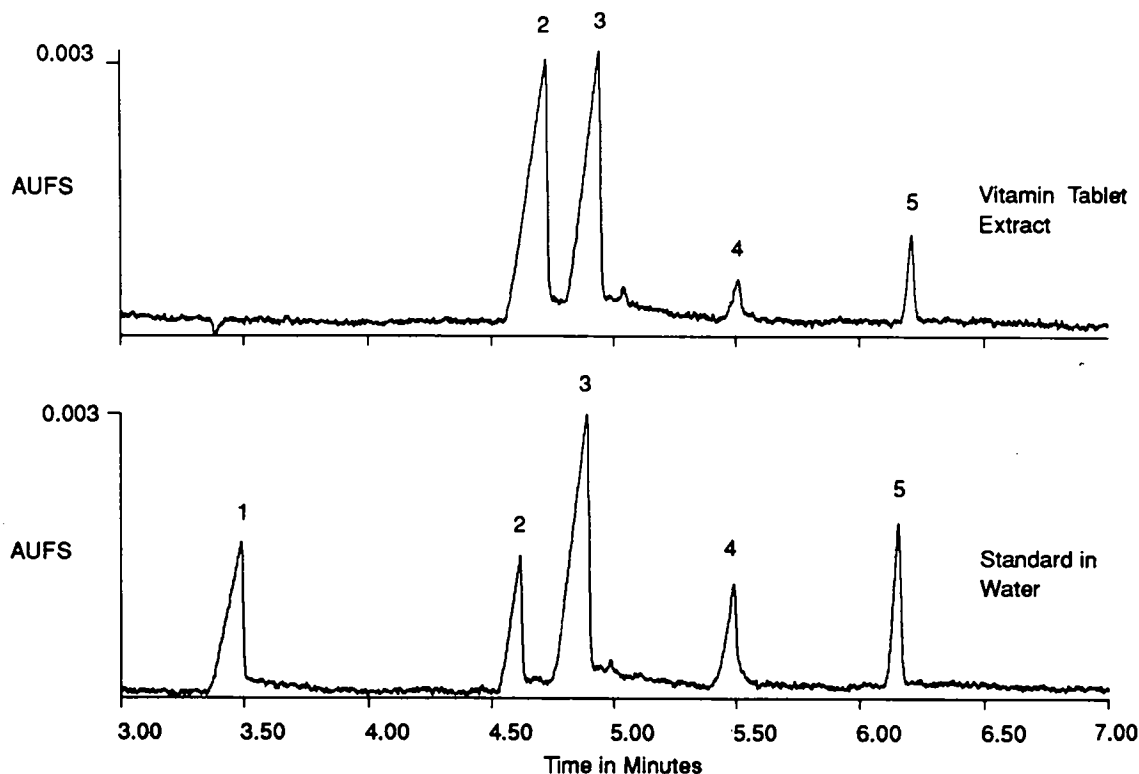
High Peak Capacity

Sub-PPM Sensitivity

Reproducible

Rapid, Simple, Highly Efficient

CIA Separation of Cations: Vitamin Tablet Extract



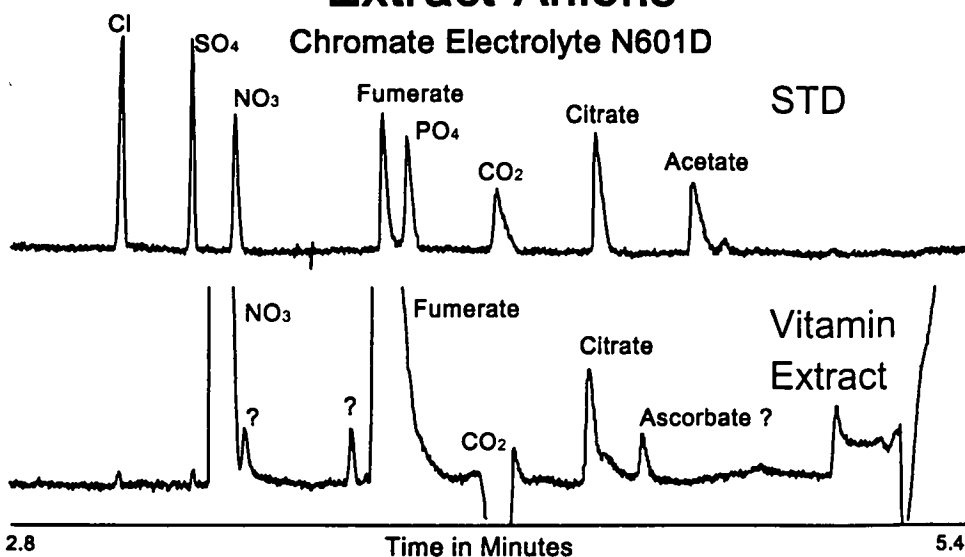
Quanta 4000 CE Conditions

Mode: CIA
Capillary: AcuSep 75 micron by 60 cm
Electrolyte: 5 mM UVCat-1, 6.5 mM HIBA, pH 4.4
Voltage: 20KV
Detection: UV @ 185 nm (indirect)
Injection: 30 second Hydrostatic
Peak ID STD: 1) Potassium 1 PPM 2) Calcium 1 PPM
 3) Sodium 1 PPM 4) Iron 3PPM
 5) Zinc 1 PPM

Vitamin Tablet Sample Prep: 1) Add tablet to 500 mL water (pH 2.0 with Nitric acid).
2) Filter and dilute 1/10 with water

Chromatogram / Electropherogram:

CIA Separation of Vitamin Tablet Extract-Anions



Objectives:

Evaluate CIA selectivity for the analysis of vitamin tablet extracts.

Details:

CIA has the selectivity, sensitivity and robustness to be used for the quantitation of active ingredients and excipients in vitamin tablet extracts.

Ordering Information:

Part Number	Description	Quantity