



# *Ion Chromatography in Pharmaceutical Drug Characterization*

*Dionex IC Users Meeting  
Providence, October 16, 2011*

*Lokesh Bhattacharyya  
Lab Chief, LACBRP  
FDA/CBER/OCBQ/DBSQC*



## *Disclaimer*

The findings and conclusions in this presentation have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any Agency determination or policy.



## *Ion Chromatography & Pharmaceutical Drugs*

- What is IC?
  - HPLC method of separation of **ionic or polar analytes** based on their interactions with **ionic functional groups** of chromatographic support and **ionic eluents**.
- **In theory**, one should be able to separate and analyze any molecule other than true apolar compounds (i.e., hydrocarbons) by IC
- **In reality**, application of to pharmaceutical drugs is limited.
  - ~110 monographs in USP-NF use IC, compared to ~4,500 total monographs
  - 2 General chapters on IC compared to ~220 total general chapters



## *Ion Chromatography & Pharmaceutical Drugs*

- Why?
  1. Traditionally, IC have been performed using dilute acid or alkali eluents. Many pharmaceutical drugs are not stable under such elution conditions.
  2. Many drug molecules have poor water solubility—requires organic solvents to dissolve.
  3. The drug molecules often contain hydrophobic surface, which interact strongly with the hydrophobic surface of the chromatographic support in strong ionic environments (acid/alkali/salt).
  4. Suppressors and electrochemical detectors do not work well in the presence of the (high) concentration of organic solvents necessary to overcome the hydrophobic interactions.
  5. It is more difficult to understand the theory of electrochemical detection compared to the theories of UV or RI detection.
  6. Historically chemists have been using reversed-phase.



## *Ion Chromatography & Pharmaceutical Drugs*

- Applications limited primarily to,
  - Ions (bisphosphonates, amines, counter-ions)
  - Molecules with extended hydrophilic surface—poor resolution by normal- or reversed-phase (i.e., aminoglycosides, sugars, sugar-alcohols)
  - Compounds that have poor solubility in organic solvents (i.e., bisphosphonates)
  - Drug molecules lacking suitable chromophores or fluorophores
- Areas of application
  - Characterization and quantitation of active drug
  - Impurity analysis
  - Counter-ion analysis—adulteration
  - Excipients
  - Dissolution testing



## *Ion Chromatography & Pharmaceutical Drugs*

Additional advantages—

- Complementary/orthogonal to other forms of chromatography and certain other analytical techniques, such as AA, ICP
  - Important method validation tool—ICH Q2
- Simple and safe effluent disposal—minimum cost
- “Green Chromatography”: Use little or no organic solvents
  - Toxic
  - Costly to buy, costly to dispose off—reduction of cost per run
  - Potential for shortage of organic solvents—shortage of acetonitrile about 2 years ago



## *Aminoglycoside antibiotics*

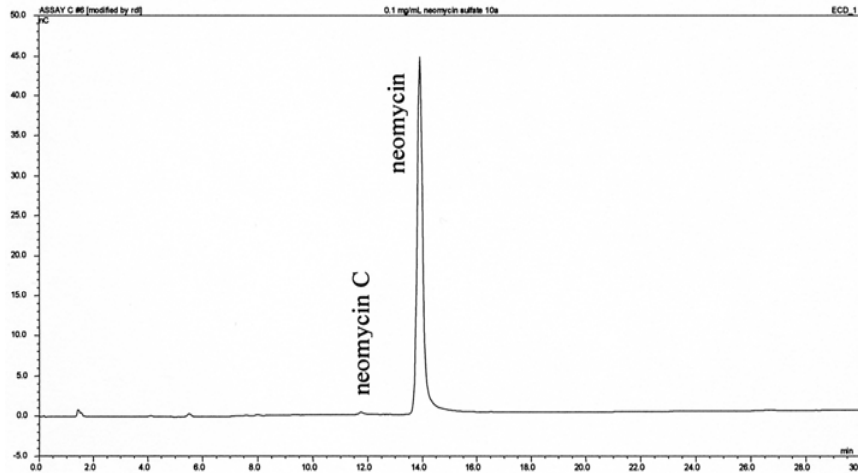
- Many antibiotics are assayed using microbial procedures
- USP-NF General Chapter <81> Antibiotics—Microbial Assay
  - Labor intensive
  - High error rate
  - Poor precision
- Aminoglycosides are carbohydrates with extended hydrophilic surfaces and no UV absorbing chromophores or fluorophores
  - Ideally suited for analysis by IC
- Objective: Develop a general IC method for quantitation of aminoglycoside antibiotics in drugs



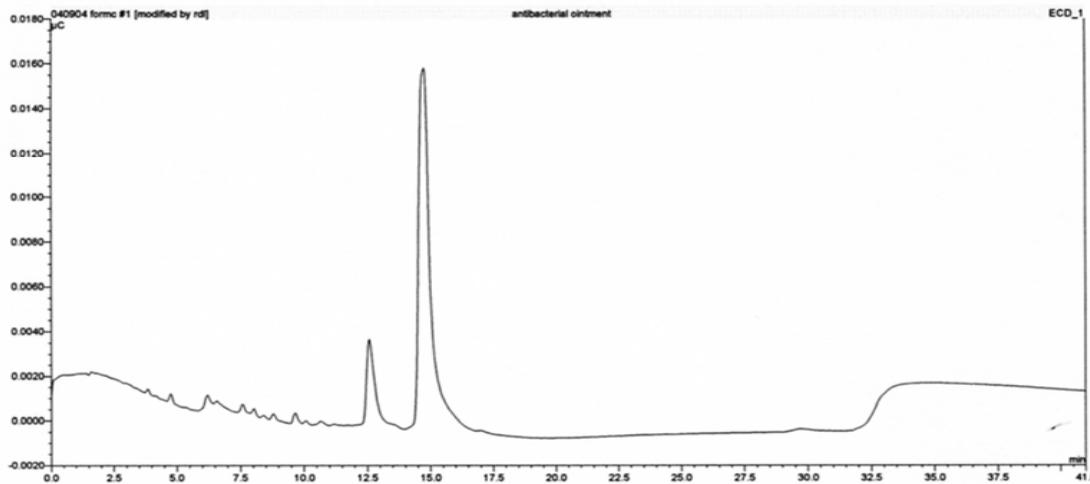
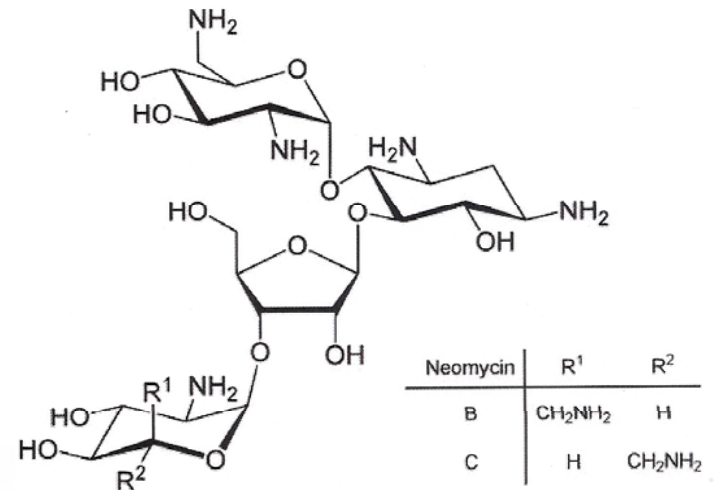
## *Neomycin*

- Chromatographic conditions
  - Column: CarboPac PA1
  - Elution: 1-5 mM NaOH, gradient; 30 min
  - Flow rate: 1.2 mL/min
  - Injection: 25  $\mu$ L
  - Temperature: 30  $^{\circ}$ C
  - Column wash with 100 mM NaOH for 10 minutes, then re-equilibration
- Sample preparation
  - Standard: USP Neomycin RS; dissolved in water
  - Sample: A topical cream containing bacitracin zinc, neomycin sulfate, and polymixin B sulfate
    - Extracted with chloroform, dried, and then dissolved in water

# Neomycin



Neomycin standard



Topical cream containing bacitracin zinc, neomycin sulfate, and polymixin B sulfate



## *Assay Parameters for Neomycin*

Repeatability (RSD) [n=6 each day over 3 days]	0.7-2.2%
Accuracy (spike recovery)	97.2-100.6%
Linearity (0.3-19.7 $\mu\text{g/mL}$ )	$\geq 0.999$
Quantitation Limit (S/N = 10)	0.3 $\mu\text{g/mL}$
Detection Limit (S/N = 3)	0.1 $\mu\text{g/mL}$
Resolution between Neomycin B and C peaks	6.5
Tailing factor:	1.5-1.8
Theoretical Plates	$\sim 21,000$



## *Neomycin Stability*

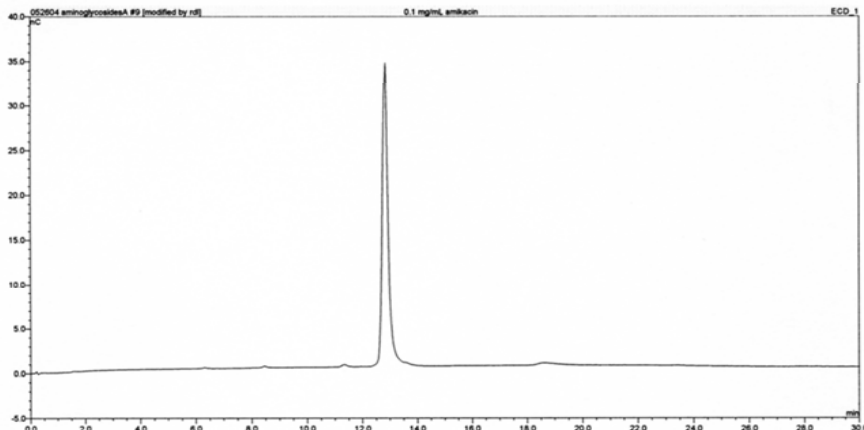
Manufacturer	Label amount* ( $\mu\text{g}/\text{mg}$ )	Experimental ( $\mu\text{g}/\text{mg}$ ) n=5
1	701	691 $\pm$ 7
2	693	734 $\pm$ 16
3@	680	595 $\pm$ 10

\*Probably by microbial assay; USP GC <81>

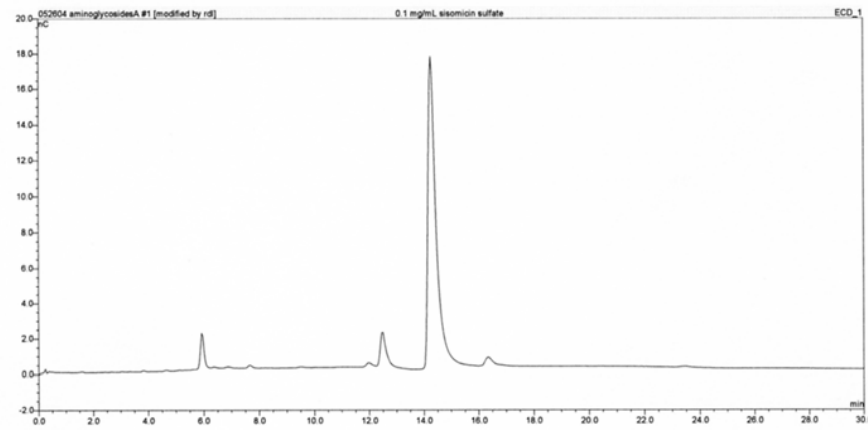
@Used 9 months after the expiration date



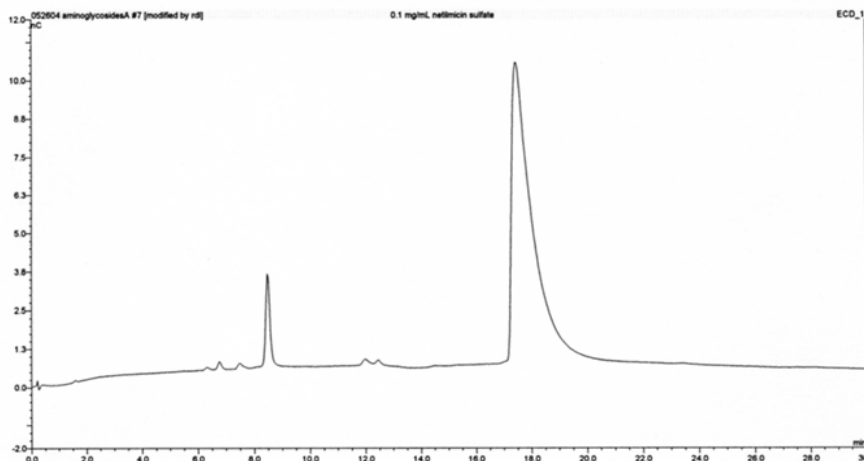
## *Other Aminoglycoside Antibiotics*



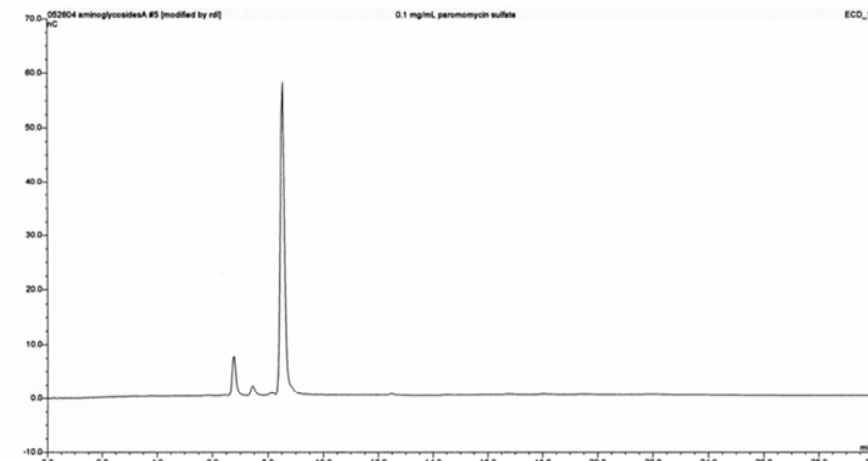
Amikacin sulfate



Sisomicin sulfate



Netilmicin sulfate



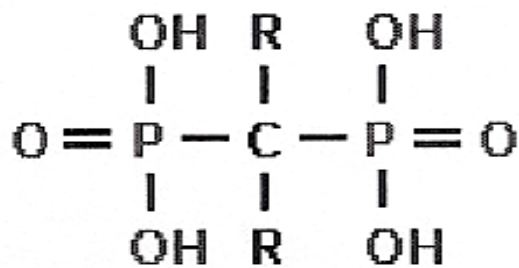
Paromomycin sulfate

## *Other Antibiotics—Assay*

- Tetracycline, oxytetracycline, chlortetracycline, doxycycline
  - Column: OmniPac PCX-100 column; Elution: 0.2 M hydrochloric acid containing 27.9% acetonitrile at  $16 \pm 2$  °C; Detection: UV 350 nm [[Ding, Mou \(2000\) J. Chromatogr. A 897, 205](#)].
- Erythromycin
  - Column: OmniPac PAX-500; Elution: NaOH/50% acetonitrile; Detection: Electrochemical, glassy carbon electrode [[USP monograph—Ointments](#)].
- Amoxicillin and flucloxacillin
  - Column: Zorbax 300-SCX; 25 mM  $(\text{NH})_4\text{H}_2\text{PO}_4$ -acetonitrile (95:5), pH 2.6; Detection: UV 225 nm [[Liu et al. \(2005\) J. Pharm. Biomed. Anal. 37, 395](#)].

## Bisphosphonate Drugs

Treatment of bone diseases: hypercalcaemia, osteoporosis, Paget's disease.



General structure

- Water soluble
- Poor solubility in organic solvents
- Poor volatility
- Anionic at low pH
- Most lack UV-absorbing chromophores

Agent	R <sub>1</sub> side chain	R <sub>2</sub> side chain
Etidronate	-OH	-CH <sub>3</sub>
Clodronate	-Cl	-Cl
Tiludronate	-H	-S-  -Cl
Pamidronate	-OH	-CH <sub>2</sub> -CH <sub>2</sub> -NH <sub>2</sub>
Neridronate	-OH	-(CH <sub>2</sub> ) <sub>5</sub> -NH <sub>2</sub>
Olpadronate	-OH	-(CH <sub>2</sub> ) <sub>2</sub> N(CH <sub>3</sub> ) <sub>2</sub>
Alendronate	-OH	-(CH <sub>2</sub> ) <sub>3</sub> -NH <sub>2</sub>
Ibandronate	-OH	-CH <sub>2</sub> -CH <sub>2</sub> -N <div style="display: inline-block; vertical-align: middle; margin-left: 10px;"> <math>\begin{array}{l} \text{CH}_3 \\ (\text{CH}_2)_4-\text{CH}_3 \end{array}</math> </div>
Risedronate	-OH	
Zoledronate	-OH	

Bisphosphonate drugs

## *Bisphosphonate Assay*

### Anion exchange chromatography

- Column
  - IC-Pak HR, PAX-100, AS series (5-11), SAX, Super-Sep IC, others
- Eluent
  - Dilute acids, pH 2-3: HNO<sub>3</sub> (2-10 mM) containing 0-20% acetonitrile, succinic acid, citric acid (z = -1)
  - NaOH or Edetate, pH 9.5 (z = -4)
- Detection
  - Indirect UV, RI, conductivity, suppressed conductivity
  - Post-column complexation with transition metal ions (Fe<sup>3+</sup>, Cu<sup>2+</sup>) and then UV
- Most assay methods are stability indicating



## *Other Drugs*

- Amines
  - Bethanechol—Assay and impurity
    - Column: IonPac CS14, Elution: 20 mM MSA, isocratic; Detection: Suppressed conductivity [[USP monograph—injection](#)].
  - Carbachol—Assay
    - Column: IonPac CS17, Elution: 5 mM MSA, isocratic; Detection: Suppressed conductivity [[Dionex AN 194](#)].
- Antiviral
  - Ribavirin (respiratory tract, hepatitis C)
    - Column: Aminex; Eluent: Dil. H<sub>2</sub>SO<sub>4</sub>, pH 2.5; Detection: UV [[USP monograph—drug substance and product](#)].
  - Foscarnet (HIV)
    - Column: IC-Pak HR; Eluent: 25 mM succinic acid; Detection: Conductivity [[Hartigh et al. \(1993\) J. Pharm. Biomed. Anal. 10, 977](#)]

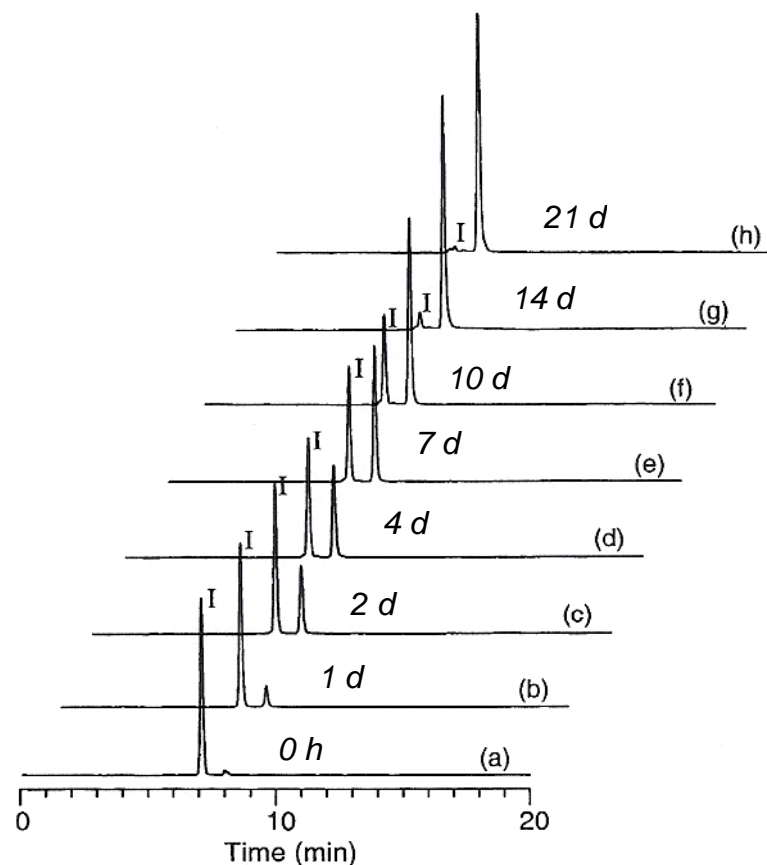


## *Other Drugs*

- Beta blocker
  - Propranolol, atenolol, metoprolol, alprenolol, oxprenolol, acebutolol
    - Column: IC-Pak CM/D; Eluent 50 mM HNO<sub>3</sub>-4% acetonitrile; Detection: UV [[Ghanem et al. \(1996\) J. Pharm. Biomed. Anal. 15,383.](#)].
- Beta-agonists
  - Salbutamol, fenoterol, clorprenaline, clenbutarol
    - Column: Metrosep Cation 1-2; Eluent: 1.8 mM HNO<sub>3</sub>-2% acetonitrile; Detection: Indirect conductivity [[Shen et al. \(2005\) J. Pharm. Biomed. Anal. 38, 166.](#)].

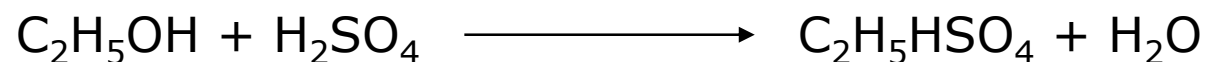
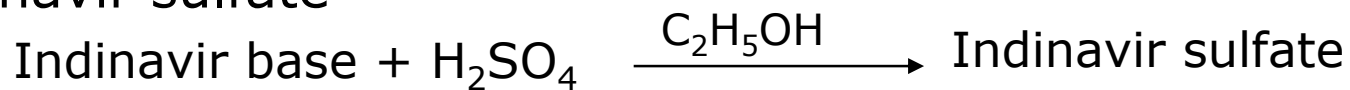
## *Impurities—Product-related*

- 2-Thioethane-1,1-bisphosphonate
- Stressed at 50 °C in dosing solution
- Chromatography
  - IC-Pak HR
  - Gradient elution 5-200 mM nitric acid-10 mM molybdate
  - Spectrophotometric detection

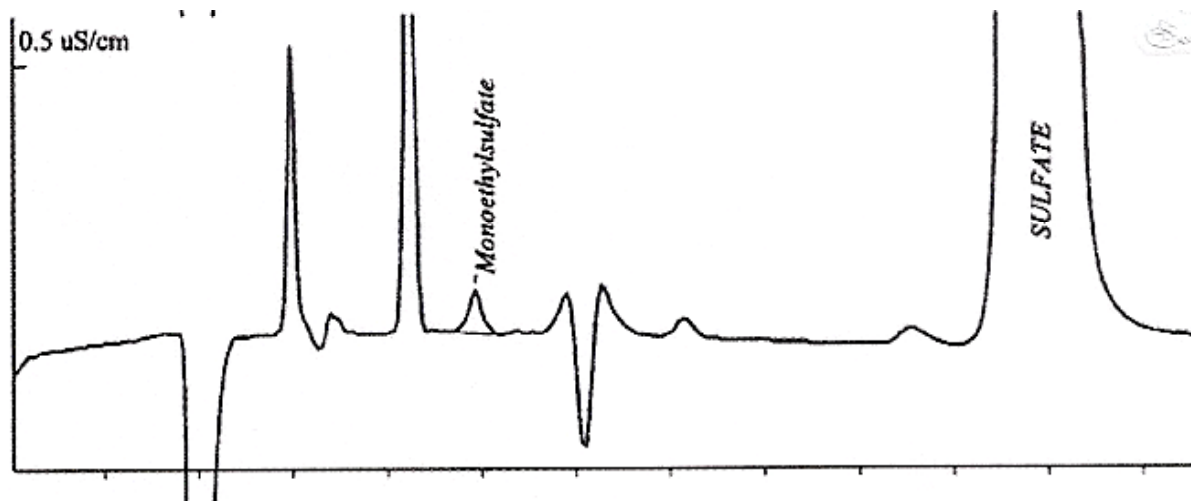


## *Impurities—Process-related*

- Indinavir sulfate



- Column: Metrosep A Supp5
- Eluent: 3.2 mM Na<sub>2</sub>CO<sub>3</sub> - 1 mM NHCO<sub>3</sub>
- Detection: Suppressed conductivity

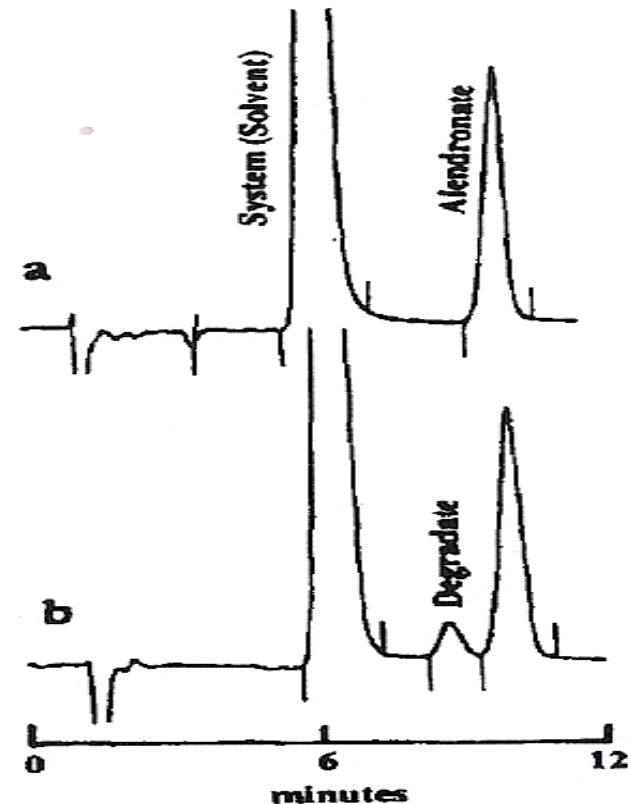


## *Drug-Excipient Interaction*

- Alendronate tablets  
Column: IC-Pak HR;  
Eluent: 1.6 mM HNO<sub>3</sub>  
Detection: Indirect UV at 235 nm

Tablets dissolved in water  
at 0.05 mg/mL alendronate

- Normal
- Stressed by incubation  
at 40 °C for 2 months



Qin et al. (1994) *J. Chromatogr. A* [686](#), 205

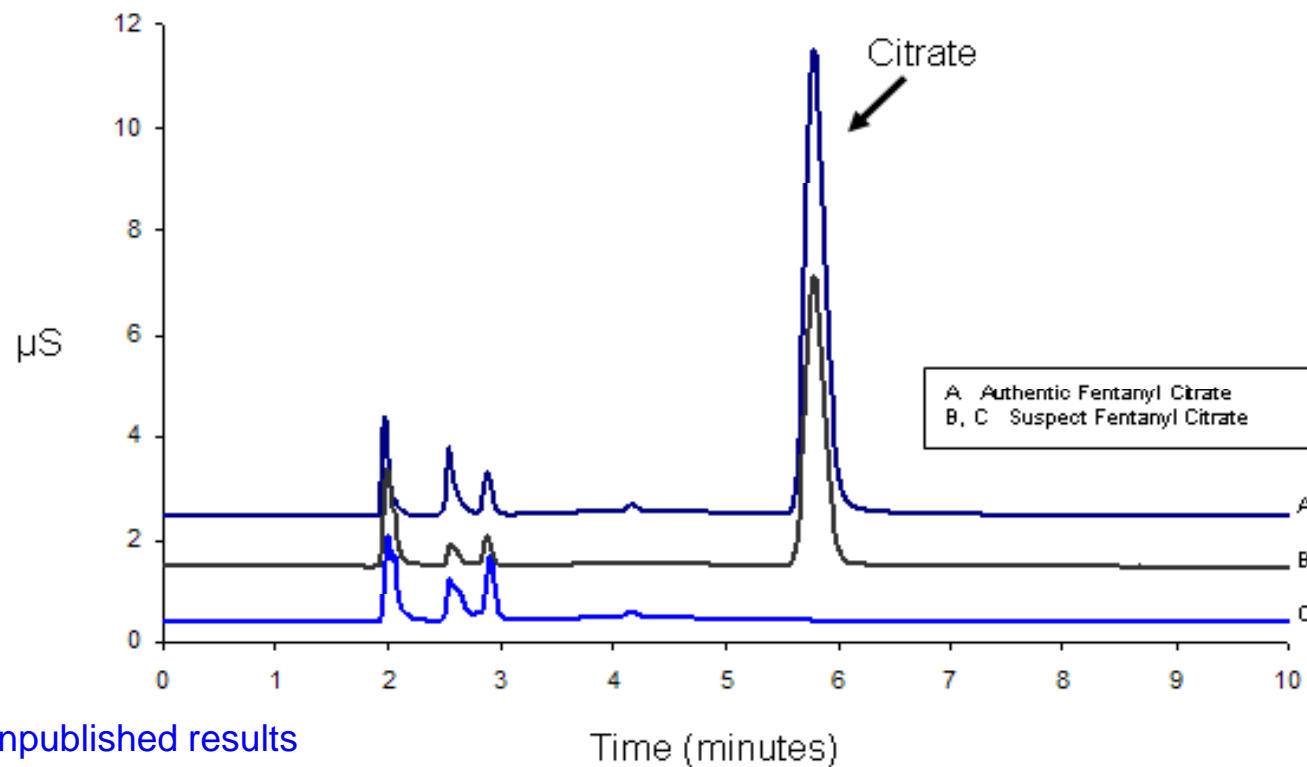


## *Counter-Ions*

- Appropriate counter-ions of active ingredients are selected to improve solubility, crystallinity, stability, and reduce toxicity, and hygroscopicity of drug substances
- Counter-ion analysis
  - To ensure stoichiometry of salt formation
  - Completeness of salt formation or salt exchange
  - Process-related impurity—residuals
  - In the investigation of drug authenticity and adulteration

## Counter-Ions

- Adulteration of Fentanyl Citrate Injection
- Column: IonPac AS11-HC; Elution: 30 mM hydroxide isocratic; Detection: suppressed conductivity





U.S. Food and Drug Administration  
Protecting and Promoting Public Health

[www.fda.gov](http://www.fda.gov)

*Thank you*

*Questions?*