A Platform Method for Pharmaceutical Counterion Analysis by HPLC

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Overview

- An analytical platform for counterion analysis by HPLC
- Simple, fast, economical analytical solution – two HPLC columns, one standard LC system with both UV and charged aerosol detector.

Introduction

Salt formation is important in drug development to improve biopharmaceutical and physicochemical properties of the drug. Approximately 50% of all drugs are formulated as salt forms. A broad selection of inorganic and organic ions can be used as pharmaceutical counterions. Because detection sensitivity is often not challenging when analyzing pharmaceutical counterions, ion analysis can be performed on an ion-exchange LC column (e.g., amino and sulfonate phases) using an HPLC system provided that a suitable detector is available. However, for such a system, anions and cations need to be analyzed separately using different methods, different separation columns, and very often, different instruments. It is highly desirable to be able to determine both pharmaceutically important anions and cations on the same separation column, using an HPLC system, and within a single analysis.

Although reversed-phase columns (e.g. C18) are most commonly used in broad range of applications, they often fail to retain highly hydrophilic molecules (e.g. counterions), and offer limited selectivities. Mixed-mode chromatography provides a viable solution to these challenges by combining both reversed phase and ion-exchange retention mechanisms. One major advantage of this approach is that column selectivity can easily be modified for optimal selectivity by adjusting mobile phase ionic strength, pH and/or organic solvent concentration. As a result, not only is the selectivity of a mixed-mode column complementary to that of reversed-phase columns, but it also allows for the development of multiple complementary selectivities on the same column under different appropriate conditions. Mixed-mode chromatography is well-suited to retaining ionic analytes, whether hydrophobic (e.g. Naproxen) or hydrophilic (e.g. Na^+ and Cl^- ions), and requires no ion-pairing agents in the method, significantly improving the MS compatibility.

This presentation will describe a platform method for screening all pharmaceutical counter ions (e.g., sodium, potassium, magnesium, calcium, chloride, bromide, nitrate, malate, citrate, sulfate, fumareate, citrate, etc). This separation solution, based on advanced column and detection technology, includes one separation column, one common mobile phase system, one LC system, and one simple chromatographic condition. Examples of pharmaceutical counter ion screening and simultaneous detection of drug substance and counter ion will be given.

FIGURE 1. Acclaim Trinity P1 and Acclaim Trinity P2

Experimental

Separation Columns

Thermo Scientific™ Acclaim™ Trinity™ P1 column (3 µm, 3 × 50 mm)

Acclaim Trinity P2 column (3 µm, 3 × 50 mm)

Samples

API and counterions were purchased from Sigma Aldrich® (St. Louis, MO, USA). Samples were dissolved in acetonitrile/water (1:1).

Mobile Phase

Acetonitrile, formic acid and acetic acid were from Thermo Fisher Scientific. High-purity ammonium acetate and ammonium formate salts were from Sigma Aldrich®.

Preparation of 0.1 M ammonium acetate (pH5.2): dissolve 7.75 g ammonium acetate and 2.00 g acetic acid in 998.0 g D.I. water.

Preparation of 0.1 M ammonium formate buffer (pH3.65): dissolve 6.35 g ammonium formate and 4.50 g formic acid in 996.0 g D.I. water.

Instruments

Separations were performed on a modular Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system equipped with an LPG 3600RS gradient pump, WPS-3000 TRS Autosampler, TCC-3200RS column oven, a PDA-3000 detector and Thermo Scientific™ Dionex™ Corona™ *ultra*™ Charged Aerosol Detector.

Data Analysis

Thermo Scientific™ Dionex™ Chromeleon™ 6.80 Chromatography Data System was used for system control and data processing.

Results and Discussion

Separation Columns

Both Acclaim Trinity P1 and Acclaim Trinity P2 columns are based on Nano-Polymer Silica Hybrid (NSH) Technology that is based on high-purity porous spherical silica particles whose inner-pore area is covalently modified with silyl ligands containing either RP/anion-exchange moieties (Trinity P1) or HILIC/cationi-exchange functionalities (Trinity P2) while the outer surface is coated with nano-polymer beads of the opposite charge by electrostatic interactions. This chemistry design creates a distinctive spatial separation of the anion-exchange and cation-exchange regions, and allows all retention mechanisms to function simultaneously and be controlled independently (see Figure 1 and Table 1 for details).

TABLE 1. Summary on Acclaim Trinity P1 and Acclaim Trinity P2

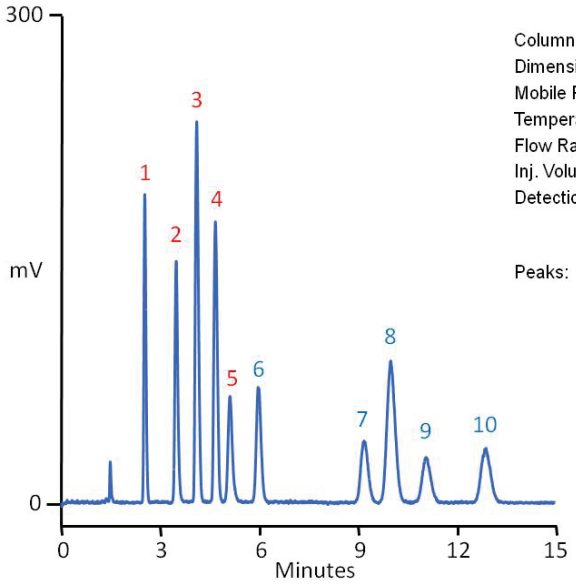
Attributes	Acclaim Trinity P2	Acclaim Trinity P1
Column chemistry	Nano-Polymer Silica Hybrid Technology (NSH)	
Retention mechanism	HILIC/WCX/SAX	RP/WAX/SCX
Counter ion analysis	Most suitable for general screening of a broad range of ions (both mono- and multi-valents)	High resolution for mono-valent ions. Some capability for multivalent ions
Simultaneous determination of API and counter ions	Good for hydrophilic APIs and counter ions	Generally good for both hydrophilic and hydrophobic APIs and respective counter ions
Other applications	Potential solution for any hydrophilic molecules, neutral or ionic, such as sugars	Generally applicable to any applications that involve ionic analytes, including both hydrophobic and hydrophilic ionics, and hydrophobic neutrals

Counterion Analysis

Figure 2 demonstrates that the Acclaim Trinity P1 column provides ideal selectivity for separating pharmaceutical counterions (including both cations and anions). Note that column selectivity is designed such that cations elute before anions.

Figure 3 illustrates that Acclaim Trinity P2 provides desired selectivity for the separation of mono- and multi-valent anions and cations – a total of twelve ions including sodium, potassium, magnesium, calcium, chloride, bromide, nitrate, malate, citrate, sulfate, fumareate and citrate are well separated on a 50-mm long column using a gradient method within 15 min. This desired feature is provided by the unique phase design in which the cation-exchange capacity and anion-exchange capacity are carefully balanced for optimal selectivity for ion separations.

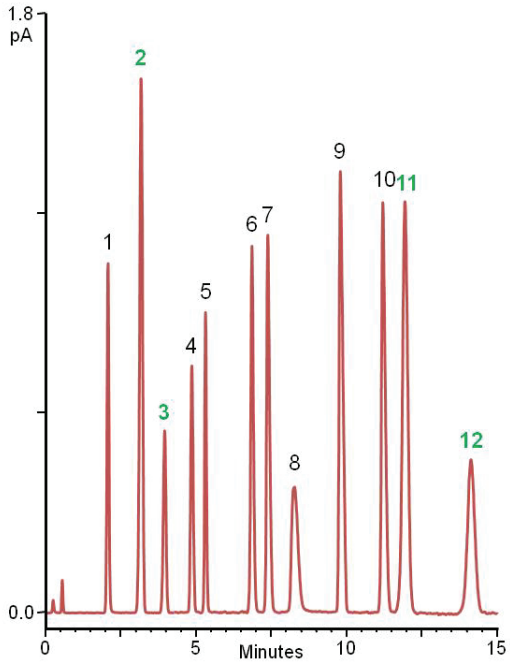
FIGURE 2. Separation of Monovalent Counterions



Column: **Acclaim Trinity P1**, 3 µm
Dimensions: 3.0 × 100 mm
Mobile Phase: 60/40 v/v CH_3CN/20 mM (total) NH_4OAc, pH5
Temperature: 30 °C
Flow Rate: 0.5 mL/min
Inj. Volume: 2 µL
Detection: Corona *ultra* (Gain = 100 pA; Filter = med; Neb Temp = 30°C)

Peaks: (50 to 100 ppm)
1. Choline
2. Tromethamine
3. Sodium
4. Potassium
5. Meglumine
6. Mesylate
7. Nitrate
8. Chloride
9. Bromide
10. Iodide

FIGURE 3. Ion (anions & cations) Screening



Column: **Acclaim Trinity P2**, 3 µm
Dimensions: 3.0 x 50 mm
Mobile Phase: D.I. water and 100 mM NH_4OFm, pH 3.65
Temperature: 30°C
Flow Rate: 0.60 mL/min
Inj. Volume: 1 µL
Detection: Corona Veo
Samples: 0.02 – 0.10 mg/mL each in D.I. water
Peaks:
1. Phosphate
2. **Sodium**
3. **Potassium**
4. Chloride
5. Malate
6. Bromide
7. Nitrate
8. Citrate
9. Fumarate
10. Sulfate
11. **Magnesium**
12. **Calcium**

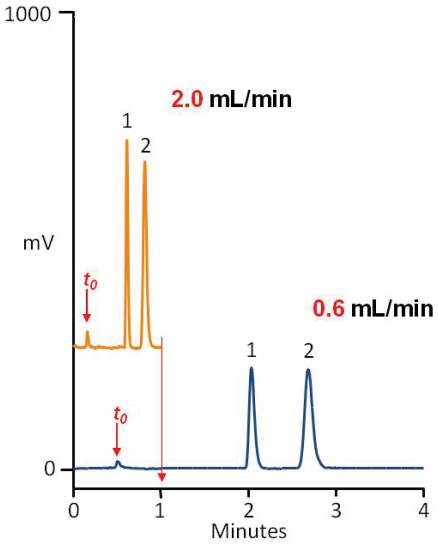
Gradient:

Time (min)	H_2O	0.1 M Ammonium formate, pH3.65
-8	90	10
0	90	10
1	90	10
11	0	100
15	0	100

Simultaneous Determination of API and Counterions

Determinations of active pharmaceutical ingredients (APIs) and counterions are important assays in pharmaceutical drug development. Due to the wide variety of charges and hydrophobicities of these pharmaceutical-related molecules, it is highly challenging to perform simultaneous separation of APIs and respective counter ions. Because of the highly hydrophilic nature of the counterion, it is impossible to assay both components within the same analysis on any RP column. On the other hand , as shown below, Both Acclaim Trinity P1 and Trinity P2 provides baseline separation of both APIs and respective counterions with excellent resolution, good peak shape, and adequate retention.

FIGURE 4. Sodium Naproxen



Column:	**Acclaim Trinity P1**, 3 µm
Dimensions:	3.0 x 50 mm
Mobile Phase:	80/20 v/v CH_3CN/20 mM (total) NH_4OAc, pH5
Temperature:	30 °C
Flow Rate:	0.6 and 2.0 mL/min
Inj. Volume:	2.5 µL
Detection:	Corona *ultra*
	(Gain = 100pA; Filter = med; Neb Temp = 30°C)
Sample:	Na, Naproxen (0.2 mg/mL in mobile phase)
Peaks:	
	1. Na^+
	2. Naproxen

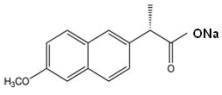
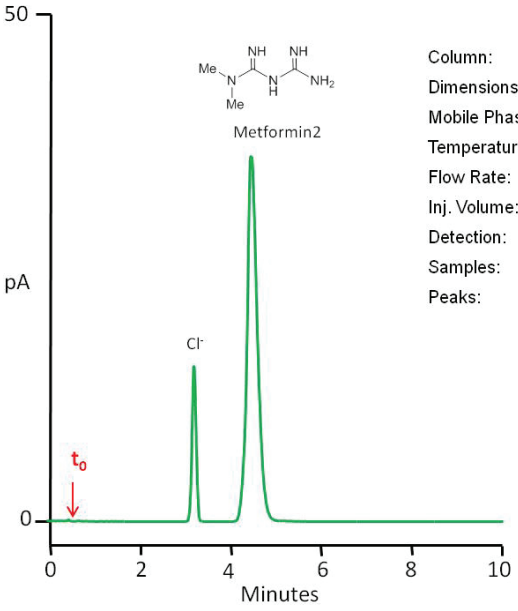
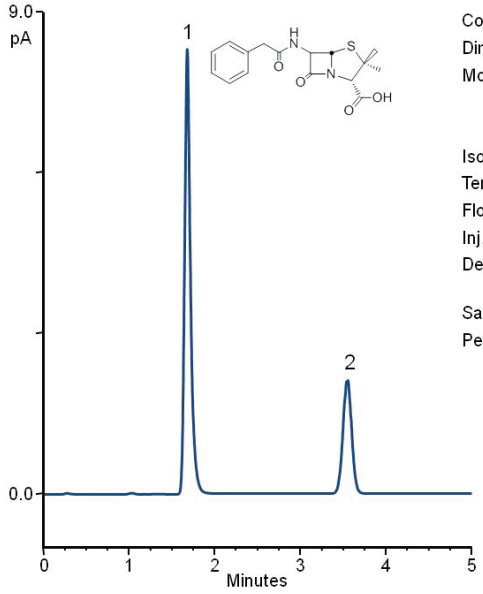


FIGURE 5. Metformin and Counterion, Cl^-



Column:	**Acclaim Trinity P2**, 3 µm
Dimensions:	3.0 x 50 mm
Mobile Phase:	MeCN /100 mM NH_4OFm, pH3.65 v/v 80/20
Temperature:	30°C
Flow Rate:	0.50 mL/min
Inj. Volume:	1 µL
Detection:	Corona Veo
Samples:	Metformin hydrogen chloride (0.1 mg/mL in D.I. water)
Peaks:	
	1. Chloride
	2. Metformin

FIGURE 6. Penicillin G Potassium Salt



Column:	**Acclaim Trinity P2**, 3 µm
Dimensions:	3.0 x 50 mm
Mobile Phase:	A: Acetonitrile
	B. Water
	C: 100 mM Ammonium formate, pH 3.65
Isocratic:	25% A / 50% B / 25% C
Temperature:	30 °C
Flow Rate:	0.50 mL/min
Inj. Volume:	1 µL
Detection:	Corona Veo: evaporator 55 °C, data rate 5 Hz, filter 2 sec, power function 1.50
Sample:	Potassium Penicillin G (0.1 mg/mL in D.I. water)
Peaks:	
	1. Penicillin G
	2. Potassium

Conclusion

Acclaim Trinity P1 and Trinity P2 columns provide an effective platform solution for pharmaceutical assays including counterion screening and simultaneous determination of API and counterion, using standard LC system and simple mobile phases.

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