

A Platform Method for Pharmaceutical Counterion Analysis by HPLC

*Xiaodong Liu, Mark Tracy, and Christopher Pohl;
Thermo Fisher Scientific, Sunnyvale, CA, USA*



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.

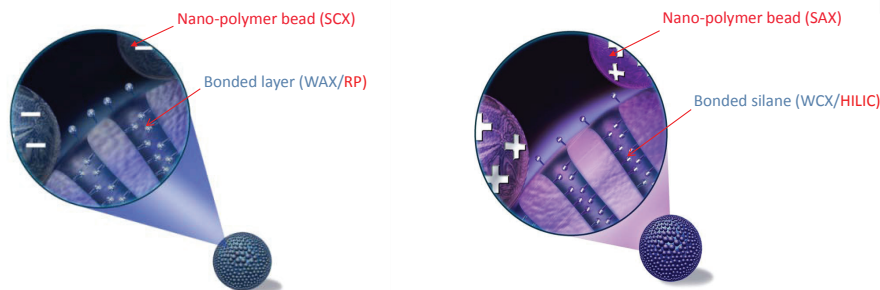
Introduction

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, fumarate, 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/cation-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, fumarate 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

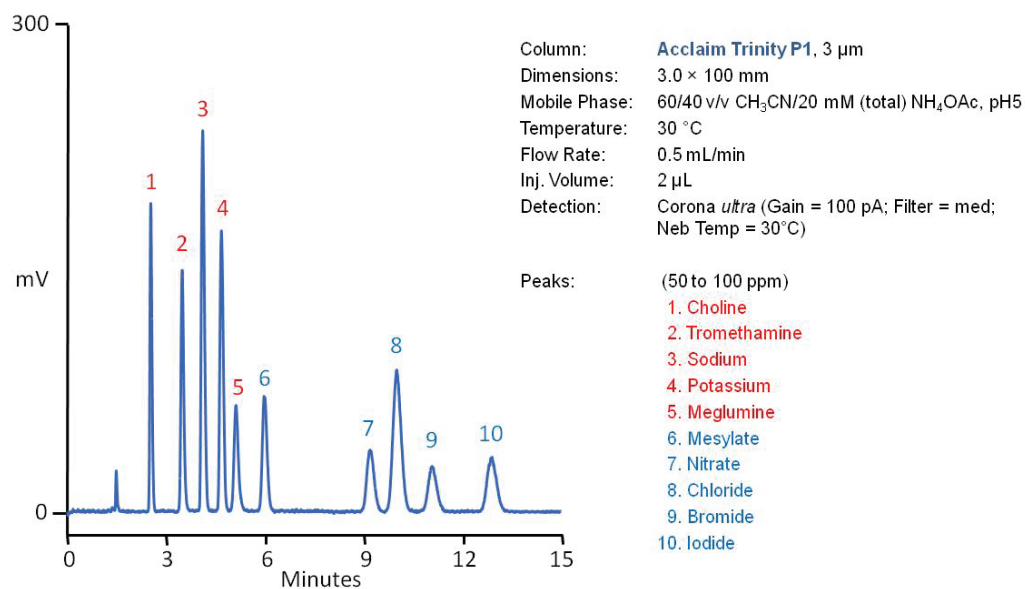
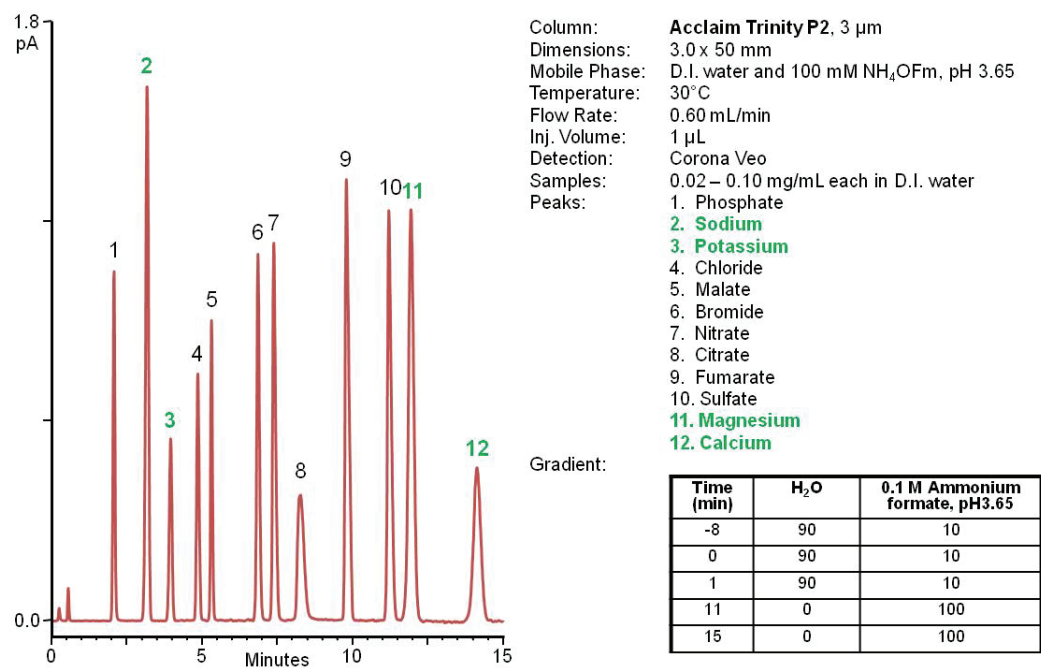


FIGURE 3. Ion (anions & cations) Screening



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

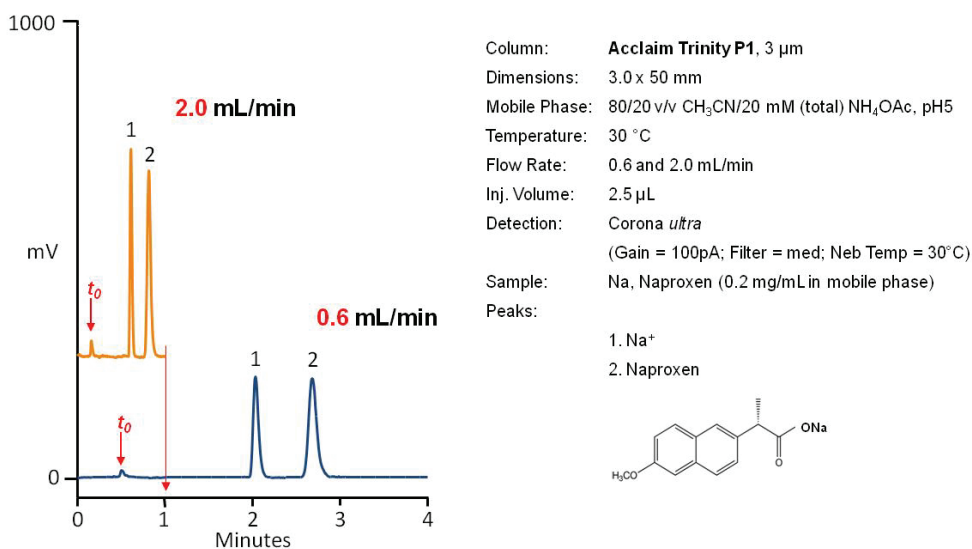


FIGURE 5. Metformin and Counterion, Cl⁻

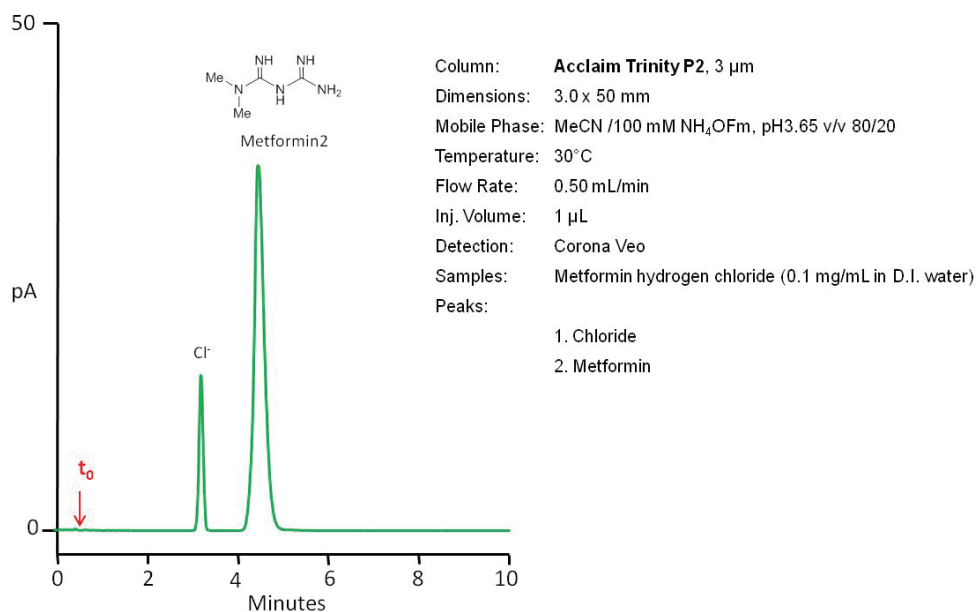
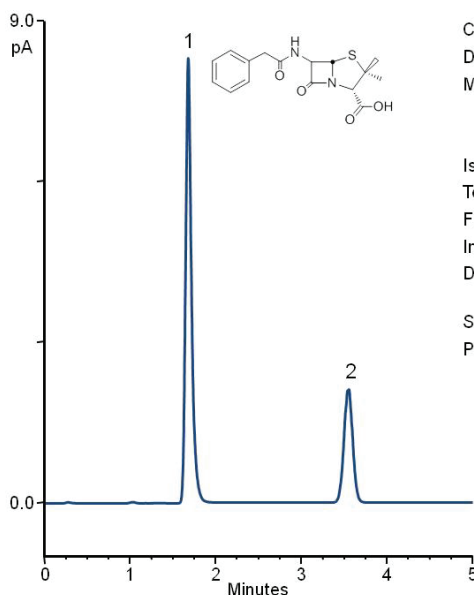


FIGURE 6. Penicillin G Potassium Salt



Column: Acclaim Trinity P2, 3 μ m
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 μ L
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.

Acknowledgements

The authors would like to thank Dr. Jinhua Chen, Mr. Yoginder Singh and Mr. Andrey Korolev for their contributions.

www.thermoscientific.com

©2014 Thermo Fisher Scientific Inc. All rights reserved. ISO is a trademark of the International Standards Organization. Sigma is a registered trademark of Sigma-Aldrich Co. LLC. All other trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries. This information is presented as an example of the capabilities of Thermo Fisher Scientific Inc. products. It is not intended to encourage use of these products in any manners that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details.



Thermo Fisher Scientific, Sunnyvale, CA
USA is ISO 9001:2008 Certified.

Africa +43 1 333 50 34 0
Australia +61 3 9757 4300
Austria +43 810 282 206
Belgium +32 53 73 42 41
Brazil +55 11 3731 5140
Canada +1 800 530 8447
China 800 810 5118 (free call domestic)
 400 650 5118

Denmark +45 70 23 62 60
Europe-Other +43 1 333 50 34 0
Finland +358 9 3291 0200
France +33 1 60 92 48 00
Germany +49 6103 408 1014
India +91 22 6742 9494
Italy +39 02 950 591

Japan +81 6 6885 1213
Korea +82 2 3420 8600
Latin America +1 561 688 8700
Middle East +43 1 333 50 34 0
Netherlands +31 76 579 55 55
New Zealand +64 9 980 6700
Norway +46 8 556 468 00

Russia/CIS +43 1 333 50 34 0
Singapore +65 6289 1190
Sweden +46 8 556 468 00
Switzerland +41 61 716 77 00
Taiwan +886 2 8751 6655
UK/Ireland +44 1442 233555
USA +1 800 532 4752

Thermo
SCIENTIFIC

A Thermo Fisher Scientific Brand