

# Separation of Biochemical Buffering Agents Using Multi-Mode Liquid Chromatography with Charged Aerosol Detection

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## Key Words

Acclaim Trinity P2, mixed-mode chromatography, Corona Veo charged aerosol detector, Good's buffers, CAD, AEX, CEX, HILIC

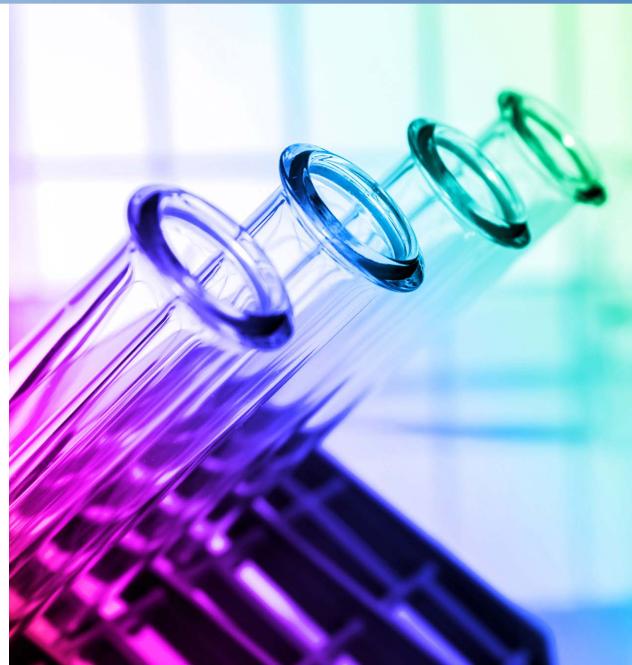
## Abstract

Good's buffers are a series of zwitterionic buffering agents popularly used in biochemical experiments and HPLC mobile phases. We present analytical conditions using the Thermo Scientific™ Acclaim™ Trinity™ P2 column to separate 22 of these and other common buffer ingredients by simultaneous anion exchange (AEX), cation exchange (CEX), and hydrophilic interaction chromatography (HILIC) with simple isocratic conditions. Since by design these buffer ingredients have low UV absorption, we use the Thermo Scientific™ Dionex™ Corona™ Veo™ charged aerosol detector.

## Introduction

Good's buffers are a series of zwitterionic buffering agents that feature stable pH control, low biological activity, low UV absorbance, and low metal affinity [1-3]. They are widely used in biological experiments and in HPLC mobile phases. Phosphate and TRIS are two other popular buffering agents. Sodium chloride is commonly used to adjust the ionic strength of buffers. As such, the need may arise to perform quality control testing of buffer formulations or residue testing after product purification. Zwitterions can be analyzed in their neutral forms by hydrophilic interaction liquid chromatography (HILIC). Ionic buffer ingredients (e.g. sodium phosphate) can be analyzed using ion exchange chromatography. Recently, LC columns have been developed that exhibit simultaneous multiple modes of retention including anion exchange (AEX), cation exchange (CEX), and HILIC, thereby providing a general approach to the analysis of buffers.

The Acclaim Trinity P2 column is based on Nanopolymer Silica Hybrid (NSH™) technology, which consists of high-purity porous spherical silica particles coated with charged nanopolymer particles. The inner-pore area of the silica particles is modified with a covalently bonded hydrophilic layer that provides weak cation-exchange retention, while the outer surface is modified with strong anion-exchange nano-polymer beads. This chemistry ensures spatial separation of the anion exchange and cation exchange regions. The Acclaim Trinity P2 column



provides ideal selectivity for monovalent and multivalent ions in highly aqueous conditions, and its hydrophilic surface makes it useful for separating hydrophilic, neutral species using HILIC-mode conditions. HILIC conditions also permit the separation of monovalent anions and cations by ion exchange.

By design, Good's buffer salts have little or no UV absorption, thus UV detection is unsuitable for their analysis. While conductivity detection is routinely used for ion analysis, it cannot be used as a general method to detect Good's buffer salts due to their small net charge. Furthermore, ions of opposite charge require different instrumental setups. Since many buffer components are nonvolatile, the Corona Veo charged aerosol detector is an excellent choice of detector. Detection begins with the nebulization of the column effluent, followed by the

generation of a dry aerosol and the subsequent adsorption of ionized nitrogen onto the particle surface. In a final step, this charge is then measured by an electrometer. With full coverage of the aerosol particles, this charge is proportional to the surface area and is thus – as a first approximation – to its mass. Since this process does not depend on the chemical or spectroscopic properties of the particles, it is nearly universal for nonvolatile analytes. Compared to light-scattering detection, Corona Veo detection provides superior detection limits, linearity, and reproducibility.

## Experimental Details

Consumables	Part Number
Thermo Scientific™ National Polypropylene vials, 1.5 mL	C4000-14 & C5000-55B
Acetonitrile, Optima™ LC/MS grade, Fisher Chemical	A955
Formic acid, Optima LC/MS grade, Fisher Chemical	A-117
Ammonium formate, Optima LC/MS grade, Fisher Chemical	A-114
Deionized water purified with Millipore® Milli-Q® Advantage A10® system	
TRIS base, POPSO disodium salt and the neutral forms of CHES, CAPS, CAPSO, MES, MOPS, MOPSO, AMPSO, Bicine, TES, ACES, TAPS, TAPSO, Tricine, HEPES, Glycine, HEPPS, and PIPES, used as received.	

## Sample Preparation

Stock solutions of the various buffering agents were prepared at 1.0 mg/mL in deionized water. Injection standards were diluted in water to 100 µg/mL.

Separation Conditions	Part Number
Instrumentation:	Thermo Scientific™ Dionex™ UltiMate™ 3000 LC system
Column:	Acclaim Trinity P2, 3 µm, 100 × 3 mm
Mobile phase	A: Acetonitrile
Mobile phase	B: 100 mM ammonium formate, pH 3.65 (6.35 g/L ammonium formate + 4.5 g/L formic acid)
Isocratic elution:	79% A, 21% B (v/v)
Flow rate:	0.60 mL/min
Column temperature:	20 °C
Injection volume:	5 µL
Detection:	Corona Veo charged aerosol detector, evaporator temperature 55 °C, gas pressure 60 psi, data rate 5 Hz, filter 2 s, power function 1.0
Column temperature:	25 °C

## Data Processing

Software:	Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System Version 6.8 SR13
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## Results

The recommended starting conditions for HILIC method development for the Acclaim Trinity P2 column use 100 mM ammonium formate buffer at pH 3.65, acetonitrile / buffer (80:20 v/v), and 30 °C. During method development, it was found that lowering the temperature to 20 °C improved the resolution of the first three peaks. To improve the resolution of chloride from MOPSO, the proportion of buffer was increased to 21%. The fixed-charge species like chloride move more slowly in response to changes to aqueous content than do zwitterions.

Figure 1 shows chromatograms of sodium, chloride, and twenty named buffering agents using simple isocratic typical HILIC conditions. Table 1 shows the corresponding CAS numbers,  $pK_a$  values, structures, and retention times. Sodium, chloride, and phosphate are ionized and retained primarily by ion exchange. Excepting phosphate and TRIS, the other eighteen buffering agents are zwitterionic. At the pH of the mobile phase, the zwitterionic agents are neutral or nearly so, and retained by hydrophilic interactions. TRIS is retained by both ion exchange and hydrophilic interaction, and therefore is the most retained.

Table 1: Buffer ingredients, their pKa, structures, and retention times under HILIC conditions using the Acclaim Trinity P2 column

Name (CAS No.)	pK <sub>a</sub>	Structure	Retention Time
CHES [103-47-9]	9.30		1.95
CAPS [1135-40-6]	10.40		2.33
CAPSO [73463-39-5]	9.60		2.76
MES [4432-31-9]	6.15		3.32
MOPS [1132-61-2]	7.15		4.03
MOPSO [68399-77-9]	6.95		4.66
AMPSO [68399-79-1]	9.0		4.77
Chloride	n.a.	Cl <sup>-</sup>	4.98
Bicine [150-25-4]	8.35		6.18
TES [7365-44-8]	7.50		6.49
ACES [7365-82-4]	6.88		6.84
TAPS [29915-38-6]	8.55		8.99
Tricine [5704-04-1]	8.15		9.72
TAPSO [68399-81-5]	7.70		10.60
HEPES [7365-45-9]	7.55		10.65
Glycine [56-40-6]	2.35, 9.60		11.28
PIPES [5625-37-6]	6.82		11.64
HEPPS [16052-06-5]	8.10		13.48
Phosphate	6.82	HPO <sub>4</sub> <sup>2-</sup>	13.48
POPSO (disodium salt) [108321-07-9]	7.85		15.58
Sodium	n.a.	Na <sup>+</sup>	19.41
TRIS (base) [77-86-1]	8.10		26.64

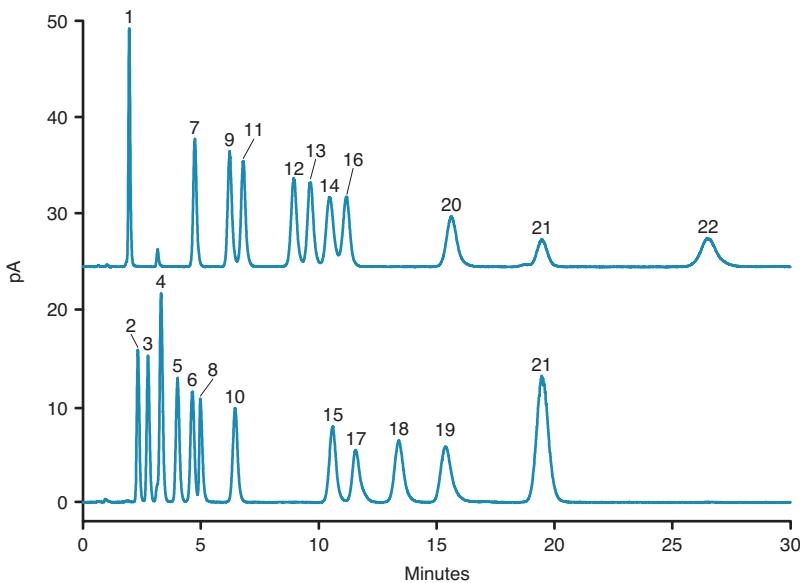


Figure 1: Separation of buffering agents using the Acclaim Trinity P2 column in HILIC mode.

Peaks: 1. CHES, 2. CAPS, 3. CAPSO, 4. MES, 5. MOPS, 6. MOPSO, 7. AMPSO, 8. Chloride, 9. Bicine, 10. TES, 11. ACES, 12. TAPS, 13. Tricine, 14. TAPSO, 15. HEPES, 16. Glycine, 17. PIPES, 18. HEPPS, 19. Phosphate, 20. POPSO, 21. Sodium, 22. TRIS.

## Conclusion

- The Acclaim Trinity P2 column offers ideal selectivity for single-run separation of zwitterions, monovalent anions, and monovalent cations using HILIC-mode conditions.
- The separation is carried out using a simple mobile phase system of acetonitrile and ammonium formate buffer.
- The Corona Veo charged aerosol detector replaces the traditional use of multiple detectors or configurations.

## References:

- [1] Good, N.E.; Winget, G. D.; Winter, W.; Connolly, T.N.; Izawa, S.; Singh, R. M. M. Hydrogen Ion Buffers for Biological Research, *Biochemistry*, 1966, 5(2), 467-477. doi:10.1021/bi00866a011. PMID 5942950.
- [2] Good, N.E.; Izawa, S. Hydrogen Ion Buffers, *Methods Enzymol.* 1972, 24, 53-68. doi:10.1016/0076-6879(72)24054-x. PMID 4206745.
- [3] [http://en.wikipedia.org/wiki/Good's\\_buffers](http://en.wikipedia.org/wiki/Good's_buffers)

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