
# HPLC Columns for Simultaneous Analysis of Counter-Ions

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Majority of the drugs are produced in a salt form. The counter ions in drugs can be inorganic and organic, acidic or basic. Depending on the property of the drug molecules, the stoichiometry can differ also. Accurate quantitation of such salts is a necessary step in pharmaceutical method development. The analysis of the drug and its counter-ion very often requires two separate methods. The task of simultaneous quantitation of counter-ions can be achieved by using mixed-mode columns. Column selection is based on the properties of the drug and the counter-ions (Table 1).

| Type of Drug Column | Basic Hydrophobic | Acidic Hydrophobic | Basic Hydrophilic | Acidic Hydrophilic |
|---|---|---|---|---|
| **Obelisc R** | Fig. 6, 7 | Fig. 5 | Fig. 9,10 | Fig. 6 |
| **Primesep D** | Fig. 1, 3 | Not recomm-ended | Not recomm-ended | Fig. 2 |
| **Primesep 100** | Not recomm-ended | Fig. 8 | Fig. 4 | Not recomm-ended |

**Table. 1 Column selection for simultaneous analysis of drug and counter ions**

There are three types of mixed-mode reversed-phase columns: bi-modal with reversed-phase cation-exchange (Primesep 100), reversed-phase anion-exchange (Primesep D), and tri-modal reversed-phase with both cation and anion-exchange properties (Obelisc R). The general approach for analysis is based on properties of both the drug and corresponding counter-ions. In mixed-mode column, compounds can be retained by reversed-phase or by the ion-exchange mechanism or their combination.

In bimodal approach the drug can be retained by reversed-phase mechanism and counter-ion by ion-exchange chromatography (Fig. 1, 3, 8). Retention of hydrophobic drugs and their elution order can be effectively adjusted by the amount of acetonitrile. Acetonitrile has a limited effect on retention of hydrophilic counter-ions, and retention for the latter can be either adjusted by the amount of buffer or by buffer pH (Fig. 3, 6, 7). Proper buffer selection is important when several hydrophilic ions need to be separated. For polycharged counter-ions, higher concentration of buffer required to facilitate timely elution. If retention is too strong, pH adjustment might be required as well (Fig. 6)

In tri-modal mixed-mode approach, three mechanisms define retention and selectivity of separation. Because tri-modal columns have both cation and anion-exchange sites along with hydrophobic chain, all mobile phase parameters can be effectively used to adjust retention time of various analytes (Fig 6, 9).

In tri-modal approach, the acetonitrile concentration will control hydrophobic retention, the mobile phase pH need to be consider since it effects ionization states of both analytes and stationary phase (pH of the mobile phase can change retention time of cations and anions in opposite direction), buffer concentration control a degree of ion-exchange interaction.

Shown examples demonstrated that the mixed-mode columns can be a versatile tool in analysis of drugs and counter-ions in one method.

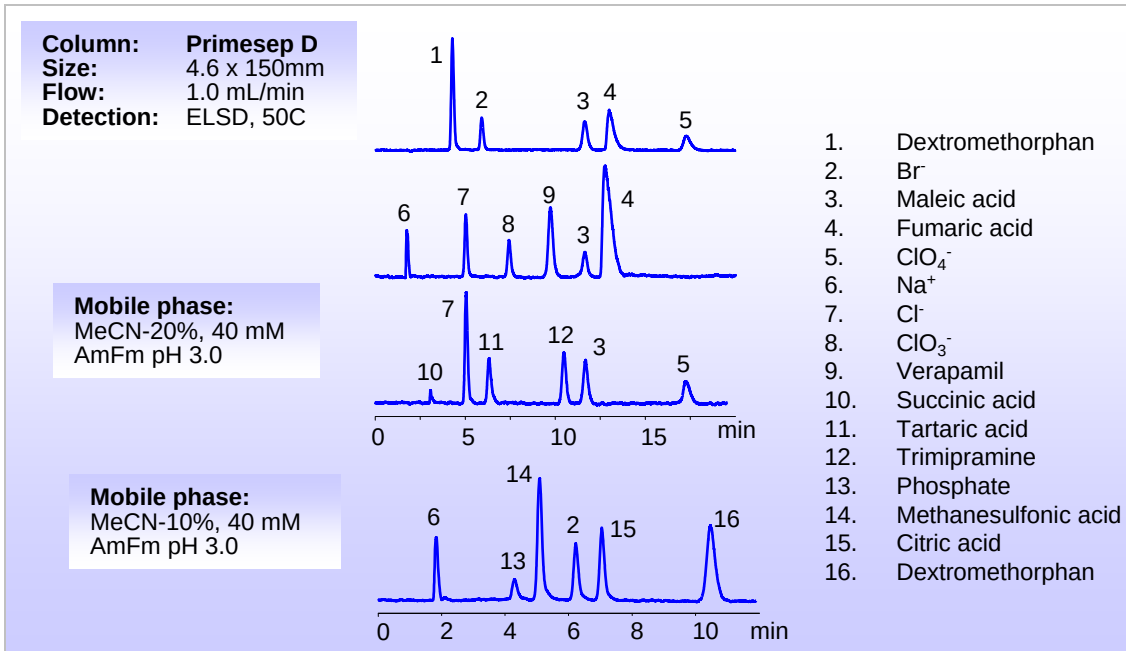


**Fig. 1 Simultaneous analysis of hydrophobic basic drugs and acidic counter-ions (bi-modal column)**

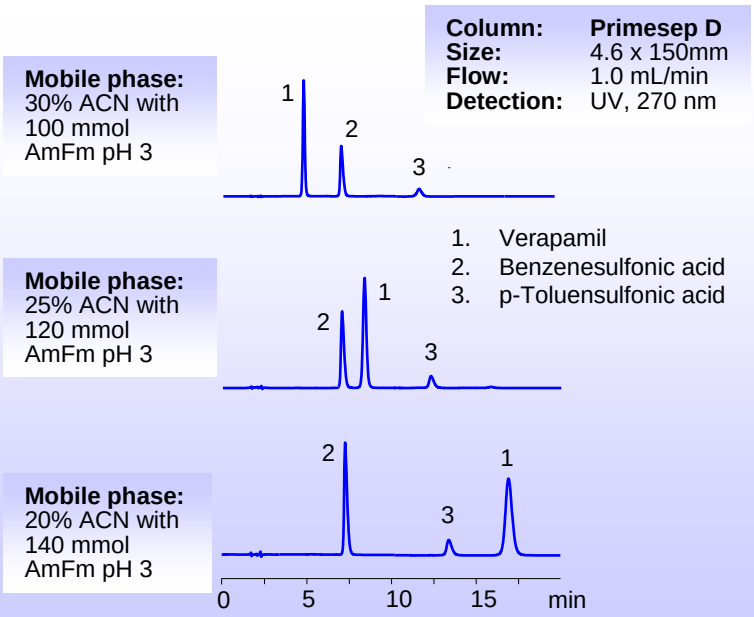


**Fig. 2 Effect of ACN Concentration on elution Order of Basic Drug and Acidic counter-Ion (bi-modal column)**

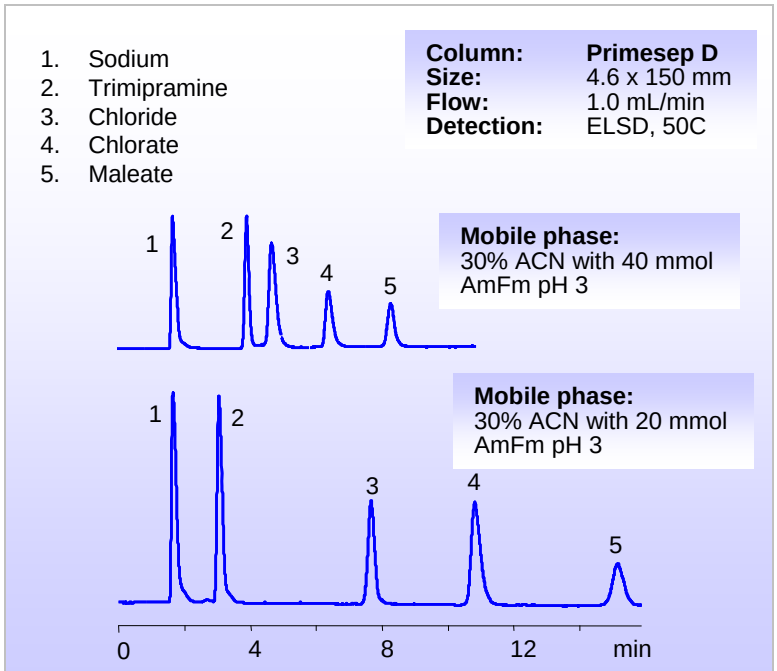


**Fig 3. Effect of buffer concentration on retention time of basic drug and counter-ions (bi-modal column)**

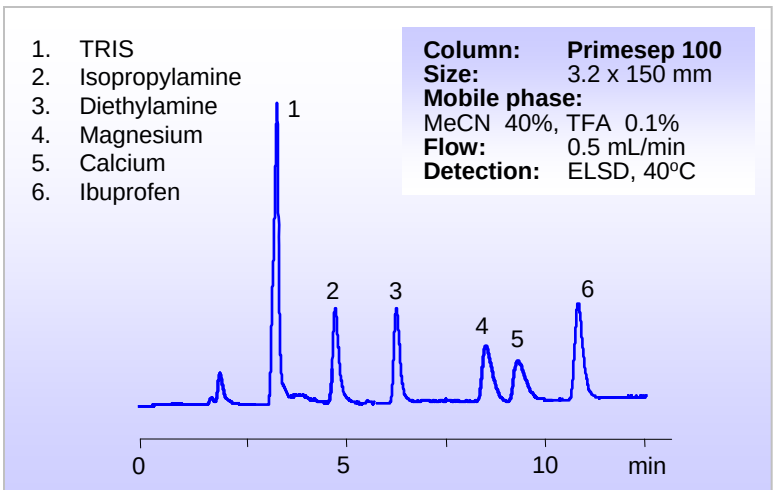


**Fig. 4 Simultaneous analysis of hydrophobic acidic drug and basic counter-ion (bi-modal column)**

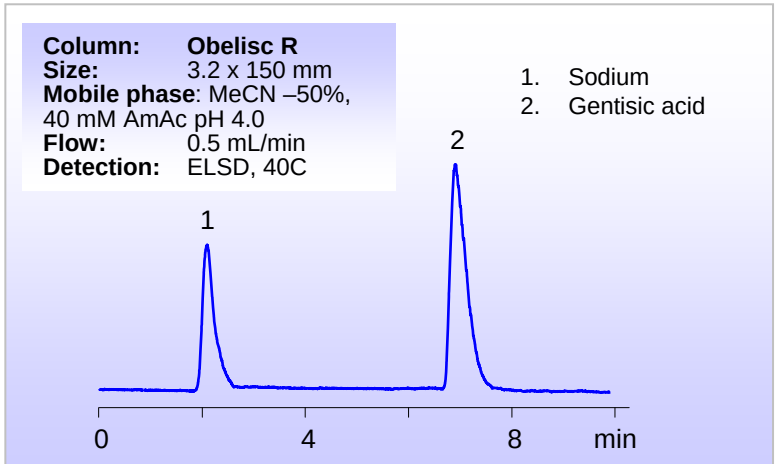


**Fig. 5 Simultaneous analysis of gentisic acidic and sodium**

1. Pseudoephedrine
2. Chlorpheniramine
3. Maleate

**Column:** Obelisc R
**Size:** 4.6 x 150 mm
**Flow:** 1.0 mL/min
**Detection:** UV 270 nm

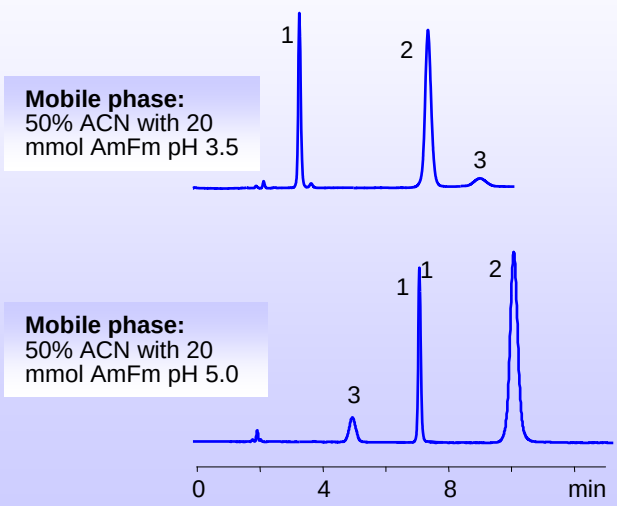


**Fig. 6 Effect of pH on elution order of basic drug and acidic counter-ion**

1. Acetaminophen
2. Pseudoephedrine
3. Dextromethorphan
4. Chlorpheniramine
5. Maleic acid

**Column:** Obelisc R
**Size:** 4.6 x 150 mm
**Flow:** 1 ml/min
**Detection:** 270 nm

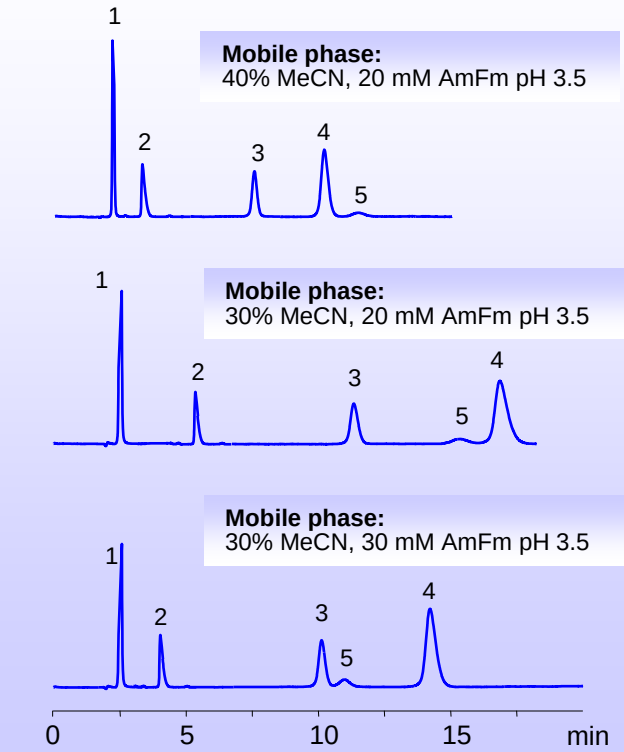


**Fig. 9 Effect of acetonitrile and buffer concentration on retention of basic drug and counter-ions (tri-modal column**

**Column:** Obelisc R
**Size:** 3.2 x 150 mm
**Flow:** 0.5 mL/min
**Detection:** ELSD, 50C

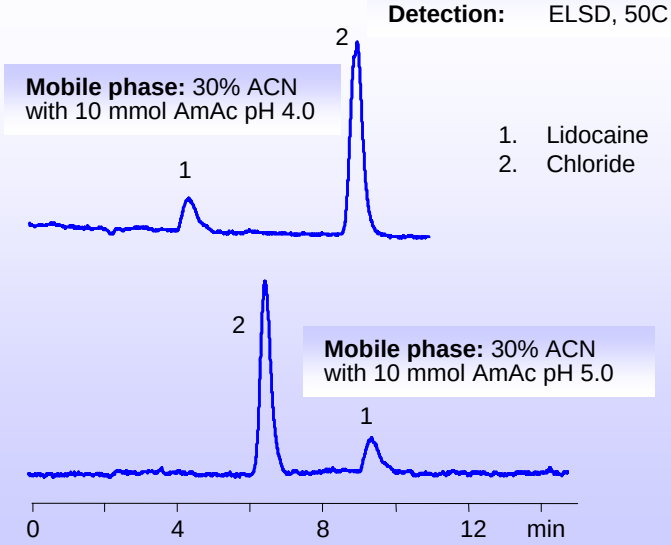


**Fig. 7 Effect of pH on elution order of basic drug and acidic counter-ion**

**Column:** Primesep 100
**Size:** 4.6x150 mm
**Flow:** 1.0 mL/min
**Mobile phase:**
50% MeCN, 0.1% TFA
**Detection:** ELSD, 50*C

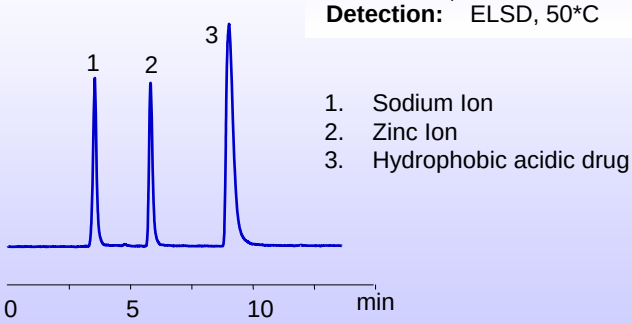


**Fig. 8 Simultaneous analysis of hydrophobic acidic drug and basic counter-ion (bi-modal column)**

1. Pseudoephedrine
2. Chloride

**Column:** Obelisc R
**Size:** 3.2 x 150 mm
**Mobile phase:**
MeCN-10%, 25 mM AmAc pH 4.0
**Flow:** 0.5 mL/min
**Detection:** ELSD, 40C

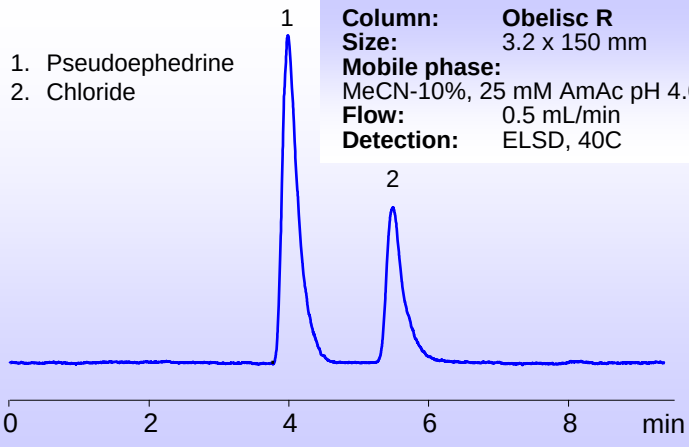


**Fig. 10 Separation of basic drug and inorganic counter-ion**