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Selective separation and purification of highly polar basic compounds using a silica-based strong cation exchange stationary phase

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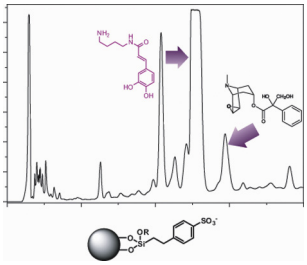
HIGHLIGHTS

- The current method is developed for very polar basic compounds.
- The symmetric peak shape of very polar basic compounds can be obtained.
- Separation conditions in SCX mode were systematically investigated.

GRAPHICAL ABSTRACT



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ABSTRACT

Compared to moderately and weakly hydrophilic bases, highly polar basic compounds are even more difficult to separate due to their poor retention in reversed phase (RP) mode. This study described the successful applications of a strong cation exchange (SCX) stationary phase to achieve symmetric peak shape, adequate retention and selectivity in the separation of very polar basic compounds. Salt and acetonitrile concentrations were adjusted to optimize the separation. Good correlations (R^2 = 0.998–1.000) between the logarithm of the retention factor and the logarithm of salt or acetonitrile concentration were obtained. Gradients generated by changing salt or acetonitrile concentration were compared for the analysis of different highly polar bases. Although all of the analytes were eluted more quickly with an acetonitrile gradient, the effect of the gradients tested on peak width and peak shape varied with respect to analyte. In addition, the effects of different types of cation and anion additives were also investigated. After separation parameters were acquired, the SCX-based method was utilized to analyze highly hydrophilic alkaloids from *Scopolia tangutica Maxim* with high separation efficiency (plate numbers > 32,000 m^{-1}). Concurrently, one very polar alkaloid fraction was purified with symmetric peak shape using the current method. Our results suggest that SCX stationary phase can be used as an alternative to RP stationary phase in the analysis and purification of highly hydrophilic basic compounds.

1. Introduction

Highly hydrophilic alkaloids, e.g. anisodine [1] and hydrophilic synthetic intermediates of basic drugs, e.g. cytosine and serotonin [2], receive much attention due to their biomedical applications. For example, serotonin is a monoamine neurotransmitter and acts as a contributor to feelings of well-being and happiness [3]. The separation approaches of such analytes will finally foster a better understanding of their bioactivity and accelerate new drug development. However, it is a challenge to separate very polar basic compounds in reversed phase liquid chromatography (RPLC) mode, since such analytes are usually peak tailing and inadequate retention [4,5].

Sometimes, the problem of poor retention can be overcome by the addition of ion-pair agents [6], the use of high pH mobile

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phase and the application of hydrophilic interaction chromatography (HILIC) [7–10]. Nevertheless, there are also some drawbacks in these approaches. For example, ion-pair agents usually increased background and caused difficulties in restoring of the initial properties of the column [11]. Most commercial silica-based stationary phases are not sufficiently stable in alkaline mobile phase [4]. The application of HILIC is usually hindered by the poor solubility of highly polar analytes in high concentration of organic solvents [12–14].

Strong cation-exchange (SCX) mode is an alternative to reversed phase and HILIC mode for high polar basic compound separation [15–18]. Although SCX mode is widely used for the analysis of biomacromolecule [19,20] and inorganic ions [21], only a few applications for the analysis of small molecule were reported. N.W. Smith and M.B. Evans did a very good job to apply ion-exchange electrochromatography for basic compound separation [22]. Luo et al. [15] developed an SCX based method to separate highly polar basic compounds. Several highly polar compounds, such as dopamine and epinephrine, were well separated by this method. However, the peak shape of basic compounds at high injection amount has not been discussed. The peak shape of amines at high injection amount was discussed in one of our previous studies [23]. The improvements of peak shape and separation efficiency were obtained for various bases. Nevertheless, this stationary phase has not been applied to separate and purify highly polar basic compounds. Many impurities in pharmaceutical industry and bioactive compounds from natural products are very polar basic compounds. For impurity analysis and bioactive compound purification, high injection amount is necessary. However, high injection amount usually lead to peak tailing. Peak tailing hinders the detection of impurities and the purification of bioactive compounds, since it results in a broader peak with a poor resolution and signal to noise. Thus, methods which can provide symmetric peak shape for very polar basic compound at high injection amount are meaningful in the fields of pharmaceutical industry and natural products.

In this study, separation conditions were optimized on an SCX column for the separation and purification of highly hydrophilic basic compounds. Parameters were adjusted including acetonitrile and salt concentration, salt type, and mobile phase gradients. Five alkaloids fractions which retain inadequately on RP columns were analyzed on SCX column. Ultimately, one hydrophilic-alkaloid fraction was purified with symmetric peak shape on the SCX column. These results indicate that the developed SCX-based method has great potential to be used as an alternative or conjunction with RPLC for the separation of highly polar basic compounds.

2. Materials and methods

2.1. Apparatus and reagents

The chromatographic system for analysis contained a 2695 HPLC pump and a photodiode array detector (PDA) system. The chromatographic system for purification contained a 2525 binary gradient pump and a 2489 ultraviolet–visible detector (UV/Vis) system. Data were collected and analyzed by Empower software version 3.0 and Masslynx software version 4.1. All instruments and workstations were purchased from Waters (Milford, USA).

The XCharge SCX columns and the XCharge C18 columns used for analysis (250 mm × 4.6 mm, 10 μm) and purification (250 mm × 20 mm, 10 μm) were purchased from Acchrom Co., Ltd. (Beijing, China). The Sunfire C18 column (150 mm × 4.6 mm, 5 μm) was purchased from Waters (Milford, USA).

Acetonitrile (ACN) and methanol were obtained from Merck (KGaA, Germany). Sodium biphosphate (NaH_2PO_4), potassium biphosphate (KH_2PO_4), ammonium biphosphate ($NH_4H_2PO_4$), sodium perchlorate ($NaClO_4$), sodium chloride (NaCl), sodium trifluoroacetate ($NaOOCF_3$) and [$^2H_1$] dimethyl sulfoxide were obtained from J&K (Hebei, China). Acetone was from Kermel (Tianjin, China). Phosphoric acid (H_3PO_4) was purchased from Tedia (Fairfield, USA). All solvents were HPLC grade and the purities of these salts were more than 98%. Water was prepared by a Milli-Q system (Billerica, MA, USA). The pH was measured by an ORION pH meter (Model 686, Thermo Fisher Scientific, USA). The electrode system was calibrated using usual aqueous standard reference buffers and all the pH readings were obtained in the pH scale before mixing the aqueous buffer and the organic modifier. For simplicity, the left side notation in pH symbols has been omitted in this paper.

The scopolamine hydrobromate trihydrate standard was purchased from Acros (NJ, USA). Cytosine, serotonin, ractopamine, epinephrine, (−)-hyoscyamine and dopamine standards were purchased from TCI (Tokyo, Japan). Anisodine was purified by our laboratory and characterized by UV, MS, IR, ^{1}H NMR and ^{13}C NMR.

The NMR spectra were measured on a Bruker Avance III 600 spectrophotometer, with [$^2H_1$] dimethyl sulfoxide as solvent. The data were dealt with MestReNova software. The mass spectra (MS) experiments were operated on obitrap LTQ-Orbitrap mass spectrometer (Thermo, San Jose, CA). Data were analyzed with Xcalibur software. The IR spectra were recorded with a Perkin-Elmer GS-II FTIR spectrometer (Perkin-Elmer, USA) in the range of 4000–400 cm^{-1}. The resolution was 4 cm^{-1} and eight scans were signal-averaged in each interferogram.

2.2. The effect of cation modifiers

The effects of different eluent cation modifiers were compared on an SCX column using ammonium biphosphate, potassium biphosphate and sodium biphosphate. Mobile phase was 30 mM salt dissolved in 50% ACN. The pH was 2.8 and flow rate was 1 mL min^{-1}. The XCharge SCX column (250 mm × 4.6 mm, 10 μm) was used for basic compounds separation. All peaks were recorded at 210 nm. Tailing factor (Tf) was measured with 5% height method according to United States Pharmacopoeia (USP).

2.3. The effect of anion modifiers

The effects of different eluent anion modifiers were compared on an SCX column using NaH_2PO_4, NaCl, $NaOOCCF_3$ and $NaClO_4$. Mobile phase was 40 mM anion modifier and 10 mM NaH_2PO_4 dissolved in 50% ACN. The pH was 2.8 and flow rate was 1 mL min^{-1}. The XCharge SCX column (250 mm × 4.6 mm, 10 μm) was used for basic compounds separation. All peaks were recorded at 210 nm.

2.4. Comparison the acetonitrile gradient and salt gradient

The compounds of anisodine, scopolamine, hyoscyamine, cytosine, serotonin, ractopamine and dopamine were analyzed by acetonitrile and salt gradients. Conditions: XCharge SCX column (250 mm × 4.6 mm, 10 μm); flow rate: 1 mL min^{-1}; mobile phase for ACN gradient is that the content of ACN shifted from 5% to 50% in 30 min, while the concentration of salt (30 mM NaH_2PO_4) and eluent pH (2.8) was constant. Mobile phase for salt gradient is that the content of NaH_2PO_4 was shifted from 5 mM to 50 mM in 30 min, while the concentration of ACN (30%, v/v) and eluent pH (2.8) was constant. All peaks were recorded at 210 nm.

2.5. Preparation of highly hydrophilic alkaloid fraction from *Scopolia tangutica Maxim*

Crude alkaloids were enriched from *S. tangutica Maxim* by an SCX-based solid phase extraction method detailed by Long et al. [24]. Then, the crude alkaloids were separated on the XCharge C18