

Article

Ion-Exclusion/Cation-Exchange Chromatography Using Dual-Ion-Exchange Groups for Simultaneous Determination of Inorganic Ionic Nutrients in Fertilizer Solution Samples for the Management of Hydroponic Culture

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Abstract: In this study, ion-exclusion/cation-exchange chromatography (IEC/CEC) using dual-ion-exchange groups (carboxy and sulfo groups) for the simultaneous determination of anions (SO_4^{2-} , Cl^- , NO_3^- , and HPO_4^{2-}) and cations (Na^+ , NH_4^+ , K^+ , Mg^{2+} , and Ca^{2+}) was developed. By using the combination of dual-ion-exchange groups, simultaneous separation of inorganic ions with HPO_4^{2-} was achieved that was impossible by the conventional IEC/CEC based on the single-ion-exchange group (carboxy group). This method was applied to the monitoring of inorganic ionic nutrients in fertilizer solution samples in hydroponic culture. As a result, a higher peak resolution of inorganic anions and cations with phosphate ion using IEC/CEC with dual-ion-exchange groups was achieved in the absence of matrix effects. In addition, the developed method helps to understand the behavior of ionic nutrients in fertilizer solution during hydroponic cultivation and is potentially useful for the individual fertilization of ionic nutrients.

Keywords: ion chromatography; simultaneous separation; ionic nutrient; fertilizer; hydroponic

1. Introduction

In 1840, German chemist J. F. Liebig reported that nitrogen, phosphate, and potassium were the three elements required for plant growth [1]. In 1913, F. Harbor and C. Bosch et al. developed a method for nitrogen gas fixation from the atmosphere, and atmospheric nitrogen has since been used as a nitrogen source [2]. In addition, atmospheric nitrogen and mineral-originated phosphorus and potassium have been used effectively as fertilizer to improve the agricultural land for stable food production in the 20th century [3].

Nowadays, hydroponics is increasingly used for cultivating several crops such as fruit vegetables and leafy vegetables because of the following advantages [4,5]:

Hydroponics is less susceptible to damage from pests than soil cultivation,

The crops can be cultivated throughout the year,

There is less injury to crops because only the fertilizer solution needs to be changed.

However, the fertilizer solution needs to be carefully monitored to maintain the ideal balance of ionic nutrients to avoid excess fertilizer or nutrient deficiency. In conventional hydroponic systems, pH and electrical conductivity (EC) glass electrodes are generally used as monitoring devices for the management of fertilizer solutions. However, it is

difficult to determine the detailed composition of ionic nutrients using the above-mentioned analyses [6–8].

Herein, the technique of ion chromatography (IC) was focused on for monitoring the composition of ionic nutrients in fertilizer solution. In a previous study, ion-exclusion/cation-exchange chromatography (IEC/CEC) was developed for the simultaneous separation of inorganic anions (SO_4^{2-} , Cl^- , and NO_3^-) and cations (Na^+ , K^+ , NH_4^+ , Mg^{2+} , and Ca^{2+}) through the single injection of a sample using a weakly acidic cation-exchange column with an acidic eluent [9,10]. This method was based on the ion-exclusion/penetration mechanism for analyte anions and the cation-exchange mechanism for analyte cations in a H^+ -formed carboxy group cation-exchange column with a weakly acidic eluent. Therefore, IEC/CEC is often applied to determine the presence and/or concentration of common anions and cations in the environmental water, such as acidic rainwater, river water, and dam lake water samples [11–13]. When an analytical sample contains phosphate ion, a visible detector based on the molybdenum-yellow or molybdenum-blue reaction is used as post-column derivatization to detect the phosphate ion individually, following the IEC/CEC separation with a conductivity detector [14,15]. However, such a system requires an additional detector (e.g., a visible detector operating at 370 or 510 nm of wavelength), one or two pumps for delivering the reagents, and a reaction coil for enhancing the colorimetric efficiency.

As part of the strategy of this study, a sulfo group ion-exchanger was applied with the carboxy group ion-exchanger, used in conventional IEC/CEC as the separation column, to obtain a better peak resolution of the inorganic anions (SO_4^{2-} , Cl^- , NO_3^-) and cations (Na^+ , K^+ , NH_4^+ , Mg^{2+} , and Ca^{2+}) with phosphate ion.

Based on the above, this study aims to: develop a high-resolution IEC/CEC using dual-ion-exchange groups (carboxy and sulfo groups) for simultaneous separation of common inorganic ions with HPO_4^{2-} without using a post-column derivatization system, demonstrate the usefulness of the developed IEC/CEC system for the monitoring of fertilizer solution used in hydroponic cultivation, and understand the timing of fertilizer addition under hydroponic cultivation.

2. Materials and Methods

2.1. Instrumentation

The developed chromatographic system consisted of dual-head plunger pumps for the eluents (LC-10AD, Shimadzu Co. Ltd., Kyoto, Japan), a column oven (CTO-10A, Shimadzu Co. Ltd., Kyoto, Japan) equipped with a manually driven injection valve (sample loop volume, 20 μL), and a conductimetric detector (CDD-10Avp, Shimadzu Co. Ltd., Kyoto, Japan) (Supplementary Figure S1). The equipment was controlled using a chromatography workstation (Chromato-PRO, Runtime Instruments, Co. Ltd., Tokyo, Japan). The flow rate of the eluents was 1.0 mL/min. The separation column temperature was set at 55 °C to maintain the lower back pressure of the columns as much as possible. An IC system (IC-2001, Tosoh Co. Ltd., Tokyo, Japan) was used in the validation step.

2.2. Separation Columns

In the system developed herein, two Tosoh TSKgel Super IC-A/C (6 mm i.d. \times 150 mm) columns packed with a polymethacrylate-based weakly acidic cation-exchanger based on the carboxy group (particle size, 3 μm ; cation-exchange capacity, 0.1 meq/mL) and a Shodex SH-1011 (8 mm i.d. \times 300 mm) column packed with polymethacrylate-based strongly acidic cation-exchanger based on the sulfo group (particle size: 6 μm ; elimination limit molecule quantity: 1000 MW) were connected in tandem as part of the separation column. The column was equilibrated with the eluent for 1 h before performing the chromatographic runs. In the validation step, the Tosoh TSKgel Super IC-AZ (4.6 mm i.d. \times 150 mm) as the anion-exchange column and TSKgel Super IC-CR (4.6 mm i.d. \times 150 mm) as the cation-exchange column were used for conventional anion-exchange and cation-exchange chromatography, respectively.

2.3. Reagents

The reagents (guaranteed reagent grade or Wako special grade) were purchased from Fujifilm Wako Pure Chemical Corp. (Osaka, Japan). Ultra-pure water (>18 MΩcm, Purelab Quest 2, Elga Veolia, High Wycombe, UK) was used to prepare the standard solutions and eluent. The sample and eluent stock solutions of MgSO₄, NaCl, NH₄NO₃, KH₂PO₄, CaCl₂, tartaric acid, and 18-crown-6 were prepared by dissolving the appropriate quantities of each solute to obtain a concentration of 0.1 M. The stock solutions were diluted with appropriate volumes of water to prepare the working solutions.

2.4. Hydroponic System

A closed hydroponic system (Green Farm UH-A01E1, Uing Co. Ltd., Osaka, Japan) was used as shown in Figure 1. In this study, *Lactuca sativa* was selected as the target crop, because of it is one of the major crops in greenhouse horticulture, including the closed hydroponic cultivation, in the world [16–18]. The fertilizer solution was prepared using 1.5 and 1.0 g/L of OAT House S1 and 2 fertilizer powders (OAT Agrio Co. Ltd., Tokyo, Japan) respectively, and diluting them with ultra-pure water. The fertilizer solution samples were collected on days 1, 4, 7, 9, 11, 14, 16, 21, 23, 25, and 28 from the hydroponic culture. After filtration through a polytetrafluoroethylene syringe filter with a 0.2 μm pore size (DISMIC[®]-25HP, Advantec Toyo Kaisha, Ltd., Tokyo, Japan), the samples were temporarily refrigerated at 5 °C, diluted 5-fold, and immediately injected into the developed IEC/CEC system.

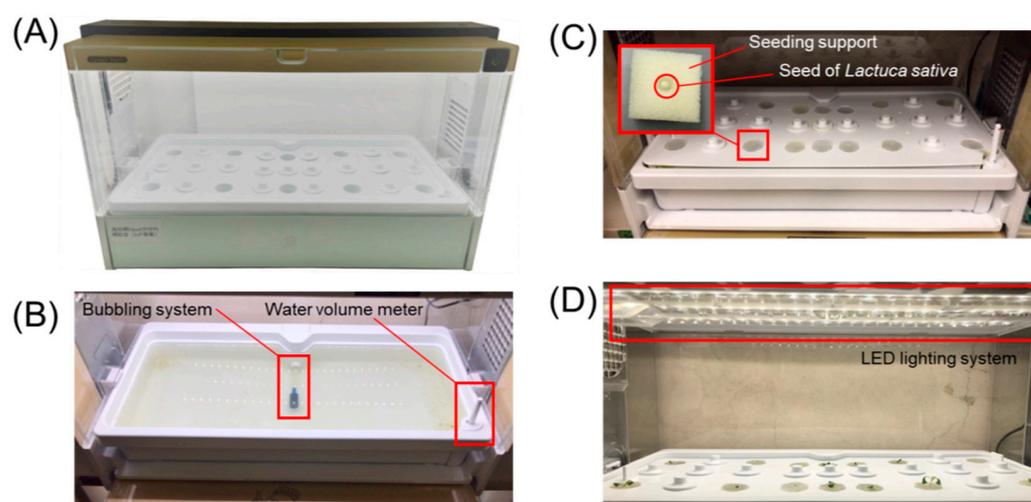


Figure 1. Closed hydroponic system used in this study. (A) Photo of the whole system, (B) storage of fertilizer solution with bubbling system and water volume meter, (C) seeding panel and support with seeds of *Lactuca sativa*, and (D) LED lighting system of the hydroponic system.

3. Results and Discussion

3.1. Selection of Separation Columns

In a previous study, a conventional IEC/CEC system was developed for the simultaneous separation of common inorganic anions and cations, excluding phosphate ion, in environmental water samples such as rain, snow, river, and dam lake water [11–13]. Using this system, the anionic species were separated through the ion-exclusion mechanism, while the cationic species were separated through the cation-exchange mechanism. A weakly acidic cation-exchange column (WCX: TSKgel Super IC-A/C) packed with a polymethacrylate-based cation-exchanger with a carboxy group as the separation column and mixed solution of tartaric acid and 18-crown-6 as the eluent were used. However, the phosphate ion peak overlapped with the Cl[−] or NO₃[−] peaks under the above-mentioned conditions, as shown in Supplementary Figure S2A.

Herein, the usefulness of a strongly acidic cation-exchange column (SCX: Shodex SH-1011) packed with a polymethacrylate-based cation exchanger with a sulfo group was demonstrated for the separation of phosphate ions from other strongly acidic anions based on the differences of penetration time of each ion into the SCX solid phase, as shown in Supplementary Figure S2B.

Based on the above chromatographic results, a combination of these two WCX columns and an SCX column was employed as the separation column to obtain a better peak resolution for phosphate and other inorganic ions. These columns were connected in tandem in this study.

3.2. Eluent Optimization

The optimization of the tartaric acid concentration in the eluent with 2 mM 18-crown-6 was conducted to achieve a good separation of analyte ions using the IEC/CEC system. As shown in Supplementary Figure S3A, the retention times of the anions were slightly increased by enhancing the penetration effects, a side effect of the IEC, into the cation-exchange phase [19,20]. In contrast, the retention times of the cations dramatically decreased by increasing the tartaric acid concentration, which, in turn, reduced the cation-exchange effect. Hence, a higher concentration of tartaric acid in the eluent should be used to reduce the analytical time as much as possible. However, when the tartaric acid concentration was increased from 2 to 8 mM, the peak resolution (R_s) between the $\text{Na}^+-\text{NH}_4^+$ (1.16–0.67) and the NH_4^+-K^+ (1.82–0.97) was inadequate, as shown in Supplementary Figure S4A. Hence, 5 mM of tartaric acid was used as the optimal concentration because it afforded the best resolution of the analyte ions under the maximum possible reduced analytical time.

Next, varying concentrations of 18-crown-6 were added to 5 mM of the tartaric acid eluent to improve the resolution between NH_4^+ and K^+ [21]. The separation is based on the stability constant of the complexation of alkali metal ions with 18-crown-6 ($\log K_{\text{Na}^+} = 0.80$, $\log K_{\text{NH}_4^+} = 1.23$, and $\log K_{\text{K}^+} = 2.03$) [22]. When included in the eluent, 18-crown-6 absorbs onto the cation-exchange resin in the column and increases the retention time of the associated monovalent cations [23]. The retention time of K^+ increased depending on the 18-crown-6 concentration, as shown in Supplementary Figure S3B. The peak resolution (R_s) between NH_4^+ and K^+ also improved when the concentration of 18-crown-6 increased in the following order: 1 mM ($R_s = 0.60$) < 2 mM ($R_s = 1.34$) < 3 mM ($R_s = 2.02$) < 4 mM ($R_s = 2.33$) < 5 mM ($R_s = 2.65$) < 6 mM ($R_s = 3.10$), as shown in Supplementary Figure S4B. The 3 mM concentration of 18-crown-6 was selected as the optimal concentration because the obtained R_s value was higher than the 1.5 required to ensure total separation.

Consequently, the IEC/CEC using 5 mM tartaric acid and 3 mM 18-crown-6 as the eluent (pH 2.98) successfully achieved simultaneous separation of the analyte ions with phosphate ion, as shown in Figure 2A. In addition, the optimized system could separate the inorganic ionic components in 5-fold diluted fertilizer solution collected on day 9 of the hydroponic cultivation, as shown in Figure 2B.

3.3. Analytical Performance

The analytical performance for the separation of the analytes under the optimized elution conditions is summarized in Supplementary Table S1. The relative standard deviation (RSD) of the retention time, peak areas, and heights of the analyte ions detected in five successive runs ranged from 0.03% to 0.04%, from 0.17% to 2.59%, and from 0.29% to 2.63%, respectively. The calibration curves of all the ions, except Cl^- , were linear in the range of 0.1–1.0 mM. The Cl^- was linear within 0.3–3.0 mM. The limits of detection (LoD) at $S/N = 3.3$ and the limits of quantification (LoQ) at $S/N = 10$ were within the range applicable to fertilizer solution samples (Supplementary Table S1). The recoveries calculated from the standard addition method by spiking the standard analytes (0.2 mM) into the fertilizer solution samples collected on day 9 of hydroponic cultivation ranged from 96.7% to 104% (Supplementary Table S1).

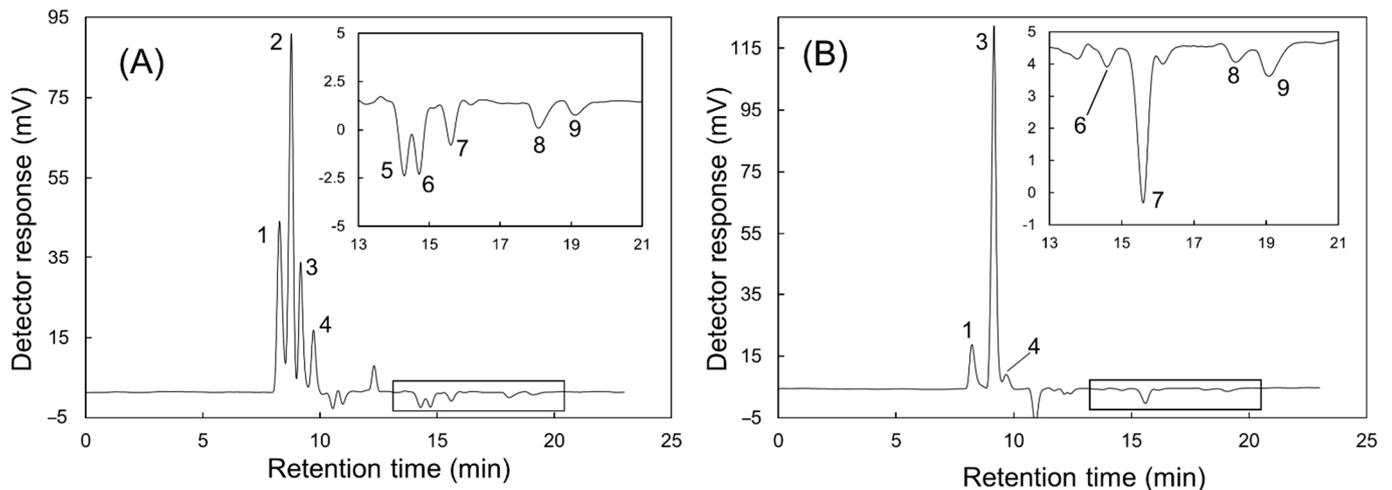


Figure 2. Optimal chromatograms for the separation of (A) standard sample, and (B) 5-fold diluted fertilizer solution sample collected on day 9. Peaks: (1) SO_4^{2-} , (2) Cl^- , (3) NO_3^- , (4) H_2PO_4^- , (5) Na^+ , (6) K^+ , (7) NH_4^+ , (8) Mg^{2+} , and (9) Ca^{2+} . Conditions: Column: Two Tosoh TSKgel Super-IC-A/C (6.00 mm I.D. \times 150 mm) and a Shodex SH-1011 (8.00 mm I.D. \times 300 mm) connected in tandem; column temperature: 55 $^\circ\text{C}$; injection volume: 20 μL ; eluent concentration: 5 mM tartaric acid and 3 mM 18-crown-6; eluent flow rate: 1.0 mL/min. Injected samples: (A) mixture of 1.0 mM MgSO_4 , NaCl , NH_4NO_3 , KH_2PO_4 , and CaCl_2 , and (B) 5-fold diluted fertilizer solution sample collected on day 9.

3.4. Monitoring the Fertilizer Solution Used in Hydroponic Culture

Fluctuations in the ionic nutrient content were simultaneously monitored using the developed IEC/CEC system during the cultivation period of *Lactuca sativa* (Figures 3 and 4). In the closed hydroponic system, the ion concentration and water volume changed, as shown in Figure 3A–C. As a result, it became difficult to understand the intake of ionic nutrients by the growing plant or that affected by the evaporation of water. Therefore, the fluctuation in the quantity of the ionic nutrients was indicated as the amount of ions (mol) present in the hydroponic solution, as shown in Figure 3B,C. The water volume gradually decreased from 4.00 L (day 0) to 1.72 L (day 28) because of evaporation and absorption by the plant (Figure 3A). On day 23, 2.00 L of fertilizer solution was added to the system after sample collection due to the expectation of the depletion of phosphate ion based on the decreasing amount of phosphate from day 21 (2.64 mol) to 23 (1.42 mol). Consequently, the water volume and the quantity of all ionic species increased on day 25 (Figure 3B,C). The height of the edible part increased moderately, and the average growing rate was 0.25 cm/day from day 0 to 11 and was 0.67 cm/day from day 11 to 28, as shown in Figures 3A and 4. All the ionic components of the fertilizer solution decreased due to plant growth. In particular, the content of the major ionic species, such as NO_3^- , K^+ , Ca^{2+} , and phosphate ions, decreased markedly from day 18 to 28 because of the acceleration in plant growth (Figure 3B,C).

3.5. Validations of Analyte Ion Concentrations

The official analytical methods for monitoring ionic species in hydroponic fertilizer solutions are not regulated. Therefore, the ionic concentrations obtained by the developed method with those determined by ion-exchange chromatography with suppressed conductivity detection, which are indicated as one of the standard methods by the EPA and JIS for monitoring inorganic ions, were compared [24,25]. The values obtained by the developed method and those obtained from the official ion-exchange chromatographic method were well-correlated ($R^2 = 0.9033\text{--}0.9894$), as shown in Supplementary Figure S5. In addition, NO_2^- was not detected in the hydroponic fertilizer samples using the standard anion-exchange chromatography technique because of nitrification in the closed hydroponic system under bubbling conditions.

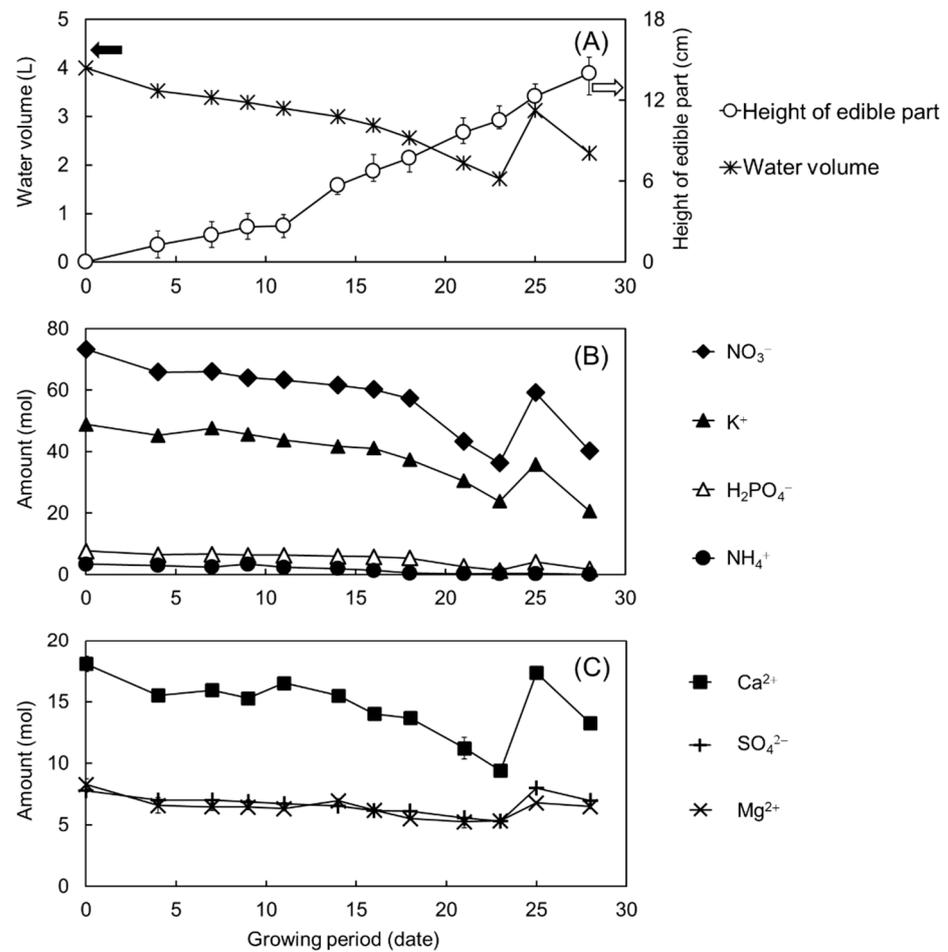


Figure 3. Variations in the height of the edible part, water volume, and amount (mol) of inorganic ionic species in the liquid fertilizer solution under the hydroponic process. (A) Height of the edible part and water volume, (B) amount of NO₃⁻, K⁺, H₂PO₄⁻, and NH₄⁺, and (C) amount of Ca²⁺, SO₄²⁻, and Mg²⁺. The experimental conditions are the same as in Figure 2. Each plot indicates the average and standard errors in three repeated measurements, except the height of the edible part (minimum and maximum values).

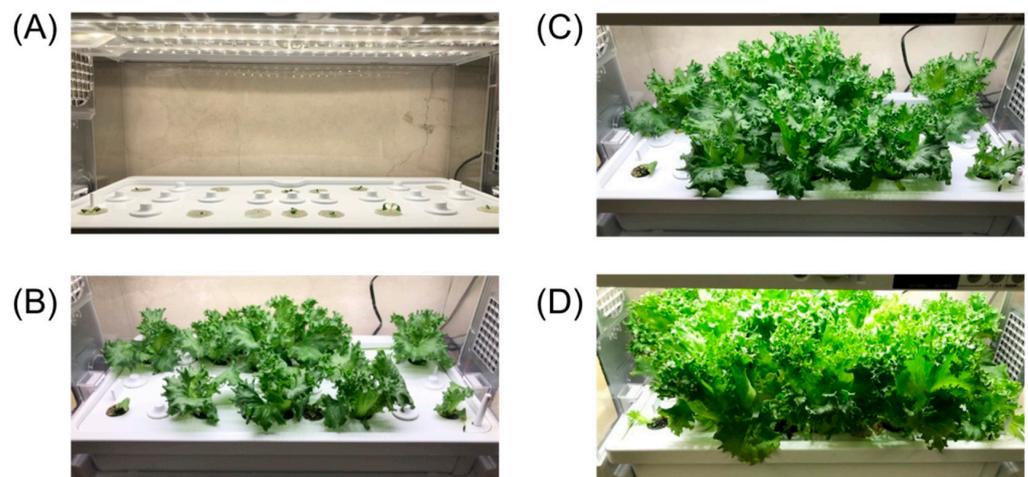


Figure 4. Photo depicting the hydroponic cultivation period for *Lactuca sativa*: (A) day 4, (B) day 14, (C) day 21, and (D) day 28.

4. Conclusions

A newly developed IEC/CEC analytical technique using dual-ion-exchange groups successfully separated and determined the phosphate ion concentration/amount along with the common anions (SO_4^{2-} , Cl^- , and NO_3^-) and cations (Na^+ , K^+ , NH_4^+ , Mg^{2+} , and Ca^{2+}) without any post-column derivatizations. This method was suitable for monitoring the ionic components in the fertilizer solution used for hydroponic cultivation. In addition, the proposed IEC/CEC technique was useful for the following:

1. It helps to understand the behavior of ionic nutrients in fertilizer solution during hydroponic cultivation.
2. It is potentially useful for the fertilization of ionic nutrients based on the understanding of all major ionic nutrients' concentration compared with the conventional monitoring system (pH and EC glass electrode).

In future research, this method can be extended to the monitoring of modest- or medium-scale hydroponic plants to understand its usefulness for the management of fertilization. It can also be applied for the management of effluent resulting from hydroponic cultivation.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/agronomy11091847/s1>, Figure S1: Schematic illustration of the proposed chromatographic system, Figure S2: Separation of analyte ions using single ion-exchange group, Figure S3: Effect of (A) tartaric acid concentration and (B) 18-crown-6 concentration on the retention time of the analyte ions, Figure S4: Effect of (A) tartaric acid concentration and (B) 18-crown-6 concentration on the resolution of Na^+ , NH_4^+ , and K^+ , Figure S5: Comparison of the analyte ion concentrations obtained from the developed method and the conventional ion-exchange chromatographic method. Table S1: Analyte performance under optimal conditions, Table S2: Analytical results for 12 fertilizer solution samples collected during the hydroponic cultivation.

Author Contributions: Conceptualization and methodology, D.K. and M.M.; validation, C.K., T.K., Y.M. and T.T.; data curation, D.K.; writing—original draft preparation, D.K.; project administration, Y.S. and T.F. All authors have read and agreed to the published version of the manuscript.

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