การหาองค์ประกอบของตัวชะให้เหมาะสมสำหรับการวิเคราะห์โลหะหนักในไลเคน

โดยวิธีไอออนโครมาโตกราฟี

DETERMINATION OF THE COMPOSITION OF ELUENT FOR ANALYZING HEAVY METALS IN THE LICHEN BY ION CHROMATOGRAPHY

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บทคัดย่อ: ศึกษาหาส่วนประกอบของตัวชะให้เหมาะสมกับการวิเคราะห์โลหะหนักในไลเคนโดยวิธีไอออนโครมาโทกราฟี คอลัมน์ที่ใช้คือ IonPacCS5A ที่มีระบบการแลกเปลี่ยนสองชนิดผสมกัน ตามปกติตัวชะที่ใช้สำหรับคอลัมน์นี้คือ กรดออกซาลิก (Ox) และไพริดีน 2,6-ไดคาร์บอกซิลิกแอซิด (PDCA) การตรวจวัดทำได้โดยวัดค่าการดูดกลืนแสง UV-Visible ของสารเชิงซ้อนที่เกิดจากโลหะที่แยกได้กับสารละลายโพสคอลัมน์ (Post column reagent, PCR) การปรับเปลี่ยนปริมาณตัวดัดแปรอนินทรีย์โซเดียมไนเตรตและลิเทียมไฮดรอกไซด์ในตัวชะกรดออกซาลิกจะมีผลต่อค่ารีเทชันไทม์และความไวของไอออนโลหะหนัก จากการทดลองพบว่าความเข้มข้นของโซเดียมไนเตรตที่เหมาะสม คือ 250 mM ในสารละลายกรดออกซาลิกเข้มข้น 28 mM ทำการปรับปริมาณลิเทียมไฮดรอกไซด์โดยใช้ 28 mM กรดออกซาลิก ผสมกับ 250 mM โซเดียมไนเตรต เป็นตัวชะ line A และ 35 mM ลิเทียมไฮดรอกไซด์ เป็นตัวชะ line B พบว่าสัดส่วนที่เหมาะสมคือ 88% A : 12% B ใช้เวลาในการวิเคราะห์น้อยกว่า 10 นาที โดยที่แมกเนเซียมและแคลเซียมที่มีอยู่ในไลเคนไม่รบกวน การวิเคราะห์ จากการทดสอบค่าขีดจำกัดของการตรวจวัด ความสัมพันธ์เชิงเส้น ความเที่ยง และความแม่น พบว่าให้ผลที่น่าเชื่อถือทางการวิเคราะห์หาปริมาณ

Abstract: Optimizing the composition of eluent for analyzing heavy metals in the lichen by the ion chromatographic method was studied. The mixed-bed ion exchange column (IonPac CS5A) was used in this study. Two commonly used eluents are oxalic acid (Ox) and pyridine 2,6- dicarboxylic acid (PDCA). The separated metals ion formed complex with the post column reagent (PCR) and detected by measuring absorbance with UV-Vis detector. The inorganic modifier sodium nitrate and lithium hydroxide affected the retention time and sensitivity of heavy metal ions. It was found that the suitable amount of sodium nitrate was 250 mM in 28 mM oxalic acid. Lithium hydroxide was adjusted by using 28 mM oxalic acid plus $NaNO_3$ 250 mM as eluent at line A and 35 mM LiOH as eluent at line B. Varying the proportion of A:B to 88% A: 12% B was the most suitable within run time of less than 10 min. Under this condition, Mg^{2+} and Ca^{2+} do not interfere with the analysis of heavy metals in lichen. The results from validation of method which involved the determination of detection limit, linearity, precision and accuracy was proved to be reliable for quantitative analysis.

Introduction: The bifunctional ion-exchange column IonPacCS5A is the most effective analytical column for heavy metals separations. The common eluents used are oxalic acid (Ox) and pyridine-2,6-dicarboxylic acid (PDCA). The selectivity of the separation is due to the different degrees of association between the metals and the chelating agent producing different net charges on the metal complex. Metal ions that can form anion complex with chelating agent in eluent are separated by anion exchange. The retention behavior mainly depends on the total complexation constant for reaction. On the other hand, some metals which form relatively weak complexes can be separated by cation exchange. By adding some inorganic compound as a modifier the selectivity, sensitivity and elution order of separation can be affected. Technical Note 10 (dionex) has used PDCA plus potassium hydroxide, formic acid and potassium sulfate as eluent and detected by measuring absorbance at 530 nm of complex formed with the post column PAR reagent. It could separate Fe^{3+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+} , Mn^{2+} and Fe^{2+}. Sutathorn et al. (2002) changed the composition of PAR, Pb^{2+} could be detected as the first ion with low detection limit. When using Ox plus potassium hydroxide and tetramethylammonium hydroxide as eluent , Pb^{2+}, Cu^{2+}, Cd^{2+}, Co^{2+}, Zn^{2+} and Ni^{2+} could be separated but it cannot elute Fe^{3+} and Cd^{2+} + Mn^{2+} coelute. In the past ten years there are many papers reported on changing the composition of PDCA and oxalic eluent, as well as studies regarding the derivertizing agent instead of commonly used PAR. Ding et.al (2000) developed the PDCA composition and used 5-Br-PADAP to form metal chelates. It was found that Pb^{2+} has higher sensitivity than using PAR and the method could detect Hg^{2+} but not Fe^{3+}.

This work aims to improve the ion chromatographic method for analyzing heavy metals in the lichens. It involves three strategies: (1) to optimize the composition of oxalic acid eluent for separating Cd^{2+} from Mn^{2+}, detect Fe^{3+} and improve detection limit of Pb^{2+}. (2) to compare the separation by using oxalic acid and PDCA eluent which separated metals form complexed with PAR and 5-Br-PADAP, and (3) to find out the condition that is suitable for analysis heavy metals in lichen samples. Lichens have been widely used as trace element atmospheric biomonitors as they are widespread and capable of absorbing element directly from the atmosphere and accumulating them in their tissue. (Scerbo et al.,1999).

Methodology: Ion chromatography equipment is a DX500 IC system, (Dionex, Sunnyvale, CA, USA), which include a quaternary gradient pump (GP50), a LC20 chromatography enclosure equipped with a Rheodyne Model 9126 injection valve with sample loop 50 μL, and an AD20 UV-vis detector. A Peaknet 5.1 chromatography workstation was used for instrument control, data collection and processing. The IonPacCS5A (Dionex) analytical column (250 x 4 mm I.D.) coupled with IonPac CG5A guard column (50 x 4 mm I.D.) was used for the separation. Eluents and all standard solutions were prepared by using deionized water, which has specific resistance > 18.0 MΩ-cm. The mixture of standard ions for analyses was prepared daily from stock standard solutions of each ion. Sodium nitrate and lithium hydroxide in oxalic acid eluent was varied the proportion to optimize the chromatographic condition for analysis of heavy metal ions (Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Fe^{3+}, Mn^{2+}, Cd^{2+} and Pb^{2+}) in lichen. Two types of eluent Ox and PDCA were evaluated the separation of heavy metals. Two types of post column reagent, PAR and 5-Br-PADAP, were used to form metal-chelate and are compared after separated by Ox and PDCA. The optimum chromatographic condition was validated in term limit of detection (LOD), linearity, precision and accuracy. Precision was determined in seven replicates and calculate in term %RSD. Accuracy of method was assessed by slope X 100 of relation between concentration added and concentration found.

Results, Discussion and Conclusion: The disadvantages of using 8.0 mM oxalic acid, 50 mM KOH and 100 mM tetramethylammonium hydroxide as eluent are as follows: 1) Mn^{2+} and Cd^{2+} were coeluted; 2) Fe^{3+} did not elute because the $Fe(OX)_3^{3-}$ complex was so stable that it was strongly adsorbed by column 3) Ca^{2+} and Mg^{2+} in large amount may precipitate in the IC system (Ding et.al.,2000). This experiment shows that $NaNO_3$ affected the elution time and stability of $Fe(OX)_3^{3-}$. Increasing $NaNO_3$ make elution of Fe^{3+}, Mn^{2+} and Cd^{2+} can be separated but Pb^{2+} was the last separated ion. When using 28 mM oxalic acid and varying the concentration of $NaNO_3$ to 50, 100, 150, 200, 250 mM (not adjust pH) with being pH about 1.68, the run time was reduced as $NaNO_3$ concentration increased. Concentration of 250 mM $NaNO_3$ gave the best resolution for all ions and the lowest run time. A typical chromatogram of a synthetic standard solution of 8 heavy metal ions is shown in Figure 1 with the run time of less than 10 minutes.

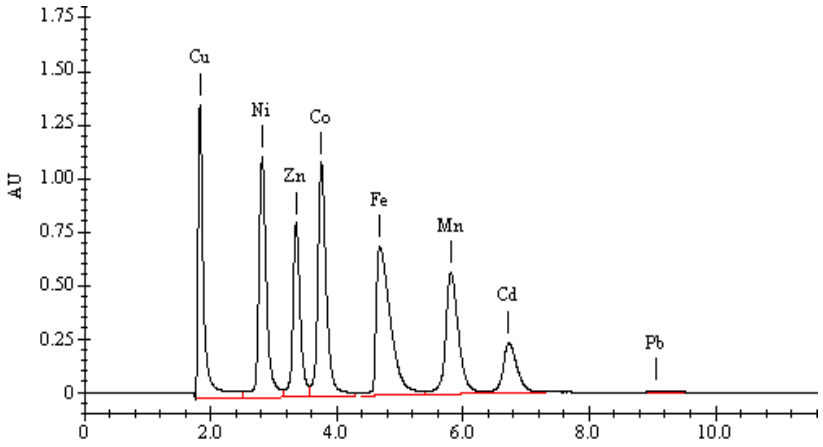


Figure 1. A typical chromatogram of a synthetic standard solution of 8 heavy metal ions by using 28 mM oxalic acid plus 250 $NaNO_3$ as eluent with PAR as post column reagent.

Accordingly to LiOH affected elution of metals especially Fe^{3+}, Pb^{2+}. Further experiment should be designed to study the effect of LiOH by making two lines of eluents. Eluent in line A is 28 mM oxalic acid plus 250 mM $NaNO_3$ and eluent in line B is 35 mM LiOH. The eluent A and eluent B were varied the proportion as %A : %B = 95:5, 96:4, 92:8, 90:10, 88:12, 86:14, 85:15 and 80:20. The chelating agent, PAR was used as post column reagent. The optimum condition was considered by elution time and peak area of Pb^{2+}. From the results it can be concluded that the optimum composition of eluent is 88% eluent A + 12% eluent B

The separation by using optimum composition of oxalic acid eluent detected by post column reagent PAR and 5-Br-PADAP were compared with the separation by using PDCA detected by post column reagent PAR and 5-Br-PADAP , the results are shown in Table 1. Using PDCA as eluent, PAR show lower sensitivity of Pb^{2+} than using 5-Br-PADAP. The latter was not suitable for oxalic acid eluent. The eluent PDCA could not detect Fe^{3+}. Lichen normally contains Fe^{3+}, so the optimum eluent should be oxalic acid. The optimum composition is 88% of A (28 mM oxalic acid + 250 $NaNO_3$) and 12% of B (35 mM LiOH) detected by PAR as PCR within run time of less than 10 minutes. Under this condition Mg^{2+} and Ca^{2+} in lichen do not interfere with the analyses. After validating the optimum chromatographic conditions, it was found that the results were appropriate for quantitative analysis. The results were shown in table 2.

Table 1. Comparison on the separation of 8 heavy metal ions by using two type of eluent and two types of post column reagent (PCR).

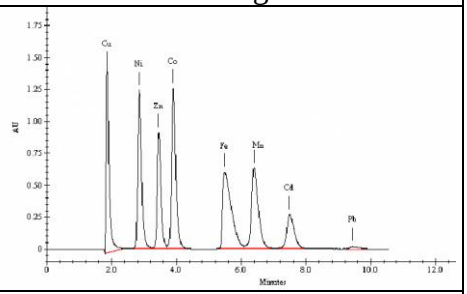
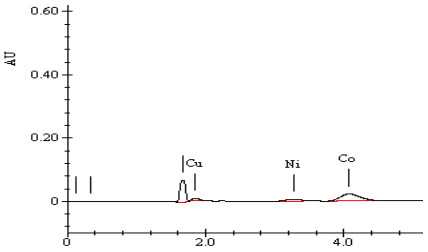
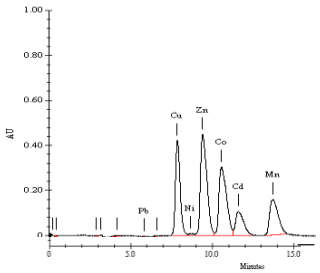
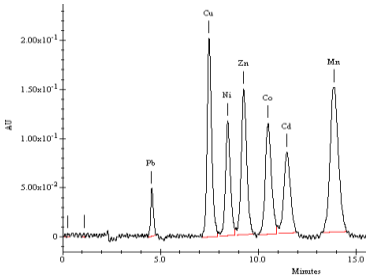
Eluent	PCR	Chromatogram
Type 1 88% (28 mM oxalic acid + 250 $NaNO_3$) + 12% (35 mM LiOH)	**Type 1** 0.3 mM PAR in 0.2 M NaOH and 0.125 M Disodium tetraborate. Detection at 530 nm	
Type 1 88% (28 mM oxalic acid + 250 $NaNO_3$) + 12% (35 mM LiOH)	**Type 2** 0.3 mM 5-Br-PADAP in 0.95% triton X-100(w/v), 0.066 M amino acetic acid, 0.067 M NaOH and 0.12 M sodium chloride. Detection at 570 nm	
Type 2 0.3 mM PDCA, 4.2 LiOH, 3 mM $Na_2C_2O_4$ and 2 mM Na_2SO_4(pH 5.1)	**Type 1** 0.3 mM PAR in 0.2 M NaOH and 0.125 M Disodium tetraborate. Detection at 530 nm	
Type 2 0.3 mM PDCA, 4.2 LiOH, 3 mM $Na_2C_2O_4$ and 2 mM Na_2SO_4 (pH 5.1)	**Type 2** 0.3 mM 5-Br-PADAP in 0.95% triton X-100(w/v), 0.066 M amino acetic acid, 0.067 M NaOH and 0.12 M sodium chloride. Detection at 570 nm	

Table 2. Limit of detection (LOD), linearity, precision and accuracy of heavy metals

Ions	LOD mg/L	Linearity		Precision %RSD (N=7)	%Accuracy
		Conc. range	r^2		
Cu^{2+}	0.011	0.25 - 4.00	0.9992	0.87	98.53
Ni^{2+}	0.008	0.25 - 4.00	0.9999	0.28	99.46
Zn^{2+}	0.013	0.25 - 4.00	0.9999	0.40	99.70
Co^{2+}	0.012	0.25 - 4.00	0.9999	0.49	99.89
Fe^{3+}	0.020	0.25 - 6.00	0.9999	2.61	99.00
Mn^{2+}	0.010	0.25 - 4.00	0.9999	0.82	99.83
Cd^{2+}	0.020	0.25 - 4.00	0.9996	1.33	102.16
Pb^{2+}	1.366	1.50 - 8.00	0.9976	5.02	86.74

Since the result shows that Pb^{2+} has the highest LOD and the lowest precision and accuracy, this method need to be further studied and improved for the analysis of Pb^{2+}.

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Keywords: Ion chromatography, heavy metals, Oxalic acid, PDCA , PAR, 5-Br-PADAP

Acknowledgements: This research is a part of the project on Production, Environmental Quality Indicator and Ecophysiology of Lichens funded by Office of the National Research Council of Thailand (NRCT). Spacial thanks are extended to Miss Tunsinee Jhampasri for her help in chemical analysis.