Inquiry Learning in Laboratory by HPLC Reversed-Phase Method Development in Taking the Conditions of Heavy Metals Separation

by Sri Wardani

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Abstract

An analysis of chromatographic method for heavy metals has been developed through formation of complex compounds with di-n-buthyldithiocarbamate. Separation of the compounds was accomplished by HPLC reversed-phase using C_{18} column and eluent of methanol-water mixture with a composition of 70:30, at a flow rate of 0.75 ml/min and UV detection at 256 nm. DBDTC complexes ofCo (II), Ni (II), Cu (II), and Hg (II) were separable in less than 10 minutes, by injecting the complexes that had been added the ligand with concentration of 0,01 M. Based on their retention times, the sequence of polarity of the metal- DBDTC complexes, in a decreasing order, is as follows: Co (II) > Ni (II) > Cu (II) > Hg (II).

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Introduction

A study about inquiry activity in laboratory has been conducted by researchers. First, the results of the study from Kipnis (2007) stated that inquiry learning in laboratory gave big opportunities for the students to do skill development and experiment process. Then, Carter, (2005) have done the laboratory activity with the results show that the students were free to do experiment and make decision about the experiment. Thus, they got the answer from their problem, had deep understanding, and were more creative. Furthermore, Blonder, Mamlok-Naaman, & Hofstein (2007) stated that inquiry activity in laboratory through open-ended could involve the students according to their skill, help the students to understand deeper and meaningful, and also there is positive correlation between the students' achievement and the results of activities in laboratory.

Laboratory inquiry activity in learning science includes chemistry, it should be done through the stages of exploration from its experience, find out and develop the supported journal, preparatory work for scientific work activity. After that, the inquiry activity begins with the observation of primary and secondary data and also involves the basic capability of scientific work, until find the conclusion as a new knowledge (Wardani, 2013, Cacciatore, 2009). Furthermore, Haryani, (2011), stated that problem-based laboratory activity could improve exploration skill in answering the problem in laboratory, and mastering the HPLC (High Performance Liquid Chromatography) material concept

HPLCmethodcould separate the heavy metals simultaneously with reversed-phase using ligand di-n-butilditiokarbamat, simultaneous separation of metallic copper (II), nickel (II), cobalt (II), using ligand di-n-butilditiokarbamat. The complex ligands formed are determined the separation using HPLC reversed-phase. in this context, the good separation condition would be traced by *trial and error* because until now there has not been patterned the solvent used yet(Wardani, 2002).

The steps of *trial and error*in looking for the separation condition using HPLC is started by investigating journals, conducting preliminary research, designing experimentto perform the separation with HPLC, discussing experimental design, experimenting the results design, then investigating journal for the second times to answer the problems that arise in laboratory. Thus, it obtained the separation condition of heavy metals using HPLC.

Laboratory inquiry activity is one approach in learning chemistry which could improve thinking skill, develop metacognition, work and communicate in team (Cacciatore (2009), Wardani (2013)). According to Rustaman (2007), in the process of learning science, it should be done through exploration of its experience, looking for and developing supported journals, designing the work steps, observing primary data involves the laboratory inquiry activity basic capability until discovering conclusion as new knowledge.

From the description above, the problem that is discussed is whether the inquiry laboratory process in laboratory could be used to find the optimum condition of heavy metals separation using HPLC?

Methodology

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The optimum condition of heavy metals separation using HPLC could be achieved by trial and error which could be carried out systematically by inquiry process as follows in Table 1.

Results and Discussion

Determination of separation condition of Co, Ni, Cu, Hg using HPLC, to obtain the optimum results should be done by investigating the literature and journals. Referral journals were able to determine type of metal complexion, type of HPLC detector, detector specification, type of eluent and its composition, eluent flow rate, so that the metals separation got the chromatogram with the good resolution and capacity factor. for optimum results, it is needed the inquiry phases:

1. Identifying the resources and designing the experiment

Analytical chemists usually do heavy metals determination by spectrophotometry or chromatography (Edward & Inatini, 1982) with the satisfying results. Ligands were organic compounds that construct complexes with the heavy metals that produce non polar complex compounds. Muller and Lovett (1985) stated that extraction method was used as concentration technique in metals analysis with HPLC, such as the metal Pd (II) determination and Rh (II) using HPLC, from the water sample after the complexity sodium dithiocarbamate (Robards, 1991).

Table 1. Inquiry Process and Separation Optimization

No	Inquiry Process/Inquiry Indicators (Alberta, 2004)	Separation Optimization Stages of Heavy Metals using HPLC
1	*Planning* phase/identifying the supported resources and designing experiment	Looking for heavy metals separation by HPLC journals and designing the experimental design
2	*Retrieving* phase/choosing the relevance information	Conducting pre-test and revising the experimental design of preliminary test results
3	*Processing* phase/choosing the exact information and recording the information	Conducting the experiment and collecting data
4	*Creating* phase/organizing the information	Analysing and presenting the data
5	*Sharing* phase/presenting and discussing the new discovery	Discussing the results
6	*Evaluating* phase/evaluating and discussing the results	Evaluating and discussing the results

The review of the literature, either through international journals, and the internet stated that the use of dithiocarbamate derivative recently is still limited to dietiditiokarbamat. Meanwhile, the other homolog such as di-n-butyl ditiokarbamat, di-n-propilditiokarbamat, and dimetilditiokarbamat, have not received proportionate attention. A number of dithiocarbamate derivatives such as di-n-butilditiokarbamat (Sidik, 1997), di-n-propilditiokarbamat (Rohani, 1998), dimetilditiokarbamat (Mulyani, 1998) have been synthesized and could be used as ions metal complexion. Based on the research results by (Jamaluddin, 2001) he stated that the metals separation of Fe, Cu, Hg with pyrolidine dithiocarbamate complexion could be done by HPLC reversed-phase with C18 column and eluent (acetonitrile:water= 66:34), the appropriate UV detector at a wavelength 254 nm.

2. Retrieving phase/Selecting the relevant information

After designing the initial planning of the experiment, then the heavy metals that will be analysed determined, they are Co, Ni, Cu, Hg, which is the heavy metals that often arise in environmental waters (Jamaluddin, 2001). The analysis of UV spectrum was performed to determine the maximum wave length of the metal complex-DBDTC, due to the simultaneous separation by reversed-phase HPLC, the UV detector was used.Separating the five-metal-DBDTC complexes was done by dissolving the respective metal complex-DBDTC in methanol. Then, the five-metal-DBDTC complexes were mixed together and added aqua bides so that the ratio of methanol: water became 70:30. in this condition, the solution of pH was 7 and the obtained peak of UV spectrum was at λ 256 nm.

3. Processing Phase/Selecting appropriate information and recording information

DBDTC metal separation, beginning with studying the reversed-phase HPLC conditions for each of the complexes Ni (II) DBDTC, Cu (II) DBDTC, Hg (II) DBDTC, and Co (II) DBDTC. Each metal complexes DBDTC attempted analysed under the same conditions, i.e. at λ 256 nm UV detector, solvent composition of methanol: water (80:20), a flow rate of 1 ml/min. The concentration of each complex metal-DBDTC is 100 ppm + 0.01 M ligand DBDTC (in a 10 mL flask). The analysis is done on an individual basis to determine the characteristic properties of each metal. Before looking alloy separation conditions. Results chromatogram peaks for each compound individually injected complex.

At the stage of selecting the appropriate information and recording the information is the stage which is doing the experiment, recording the result, and also remaining to correct the procedure in case the result of chromatogram in separation of DBDTC metals was not good. The DBDTC metals separation began with studying the reserved phase HPLC conditions for each of the complexes Ni (II) DBDTC, Cu (II) DBDTC, Hg (II) DBDTC, and Co (II) DBDTC. Each DBDTC metals complexes was tried to be analysed in the same condition which is inat λ 256 nm UV detector, solvent composition of methanol: water (80:20), a flow rate of 1 ml/min. The concentration of each metal-DBDTC complexes was 100 ppm + 0.01 M ligand DBDTC (in a 10 mL flask). The analysis is done on an individual basis to determine the characteristic of the properties in each metal. The top result of chromatogram for each complex compounds which is injected individually can be seen in the Figure 1 and 2 as follows.

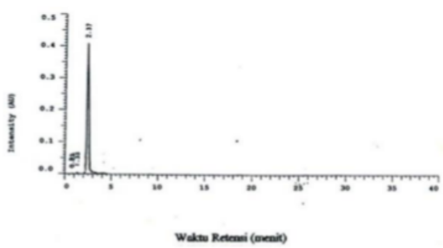

Figure 1. The Chromatogram for the complex compound Ni (II) DBDTC with the eluent composition of methanol:water (80:20) and the flow rate 1 ml/minutes

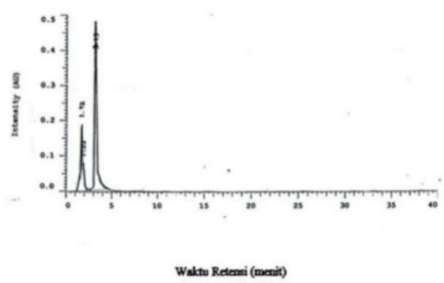

Figure 2. The Chromatogram for the complex compound Cu (II) DBDTK with the eluent composition of methanol:water (80:20) and the flow rate 1 ml/minutes

To obtain good separation conditions on the mixture of metal-DBDTC complexes, it was required changes of eluen composition towards the increasing of the polarity which is the ratio of methanol:water (70:30). The metal-DBDTC complex compounds that have a relatively high hydrophobicity will be retained in the stationary phase and the flow rate slowed to 0.75 ml/min in order to be a better separation.

Table 2. The Retention Time of metal-DBDTC metal Complex Compounds with Eluent Composition of Methanol:Water (80:20) in a Flow Rate of 1 ml/min

No.	The Concentration of Complex Componds 100 ppm + 0,01 M DBDTC	Retention times (Minutes)	
		Metal-DBDTC	Ligand-DBDTC
1.	Ni (II) DBDTC	2.37	0.82
2.	Cu (II) DBDTC	3.19	1.72
3.	Hg (II) DBDTC	3.93	1.64
4.	Co (II) DBDTC	1.98	0.83

4. Creating Phase/Organizing information

The analysis results of metal DBDTC individually produced the retention time of each metal, besides that, it also improved the eluent composition into methanol: water (70:30), with the eluent composition towards the increasing of polarity. The analysis of a mixed-metal-DBDTC complex compounds were determined from the optimization of HPLC conditions for the individual analysis of metal-DBDTC complex compounds above. Thus, for the separation of individual mixture of metal- DBDTC complex compounds, it was set that λ of UV detector was at 256 nm, the eluent composition of methanol: water (70:30) and the flow rate of 0.75 ml/min. Each metal-DBDTC complex had a concentration of 100 ppm.

The number of DBDTC ligands added in the mixture while searching for this separation of the reserved phase HPLC conditions, it was started with the mixture of 2 metal-DBDTC complexes up to the mixture of 4-metal DBDTC complexes. With the variation of the DBDTC ligand concentration added, apparently it gives a shift of retention time for each type of metal-DBDTC complex. The shift of the retention time was possible because the retention time had a characteristic but not specific. The same components might have different retention times, this can be caused a change of the number of metal-DBDTC separated, solvent composition and flow rate of eluent.

5. Sharing Phase/Presenting and discussing new discoveries

This stage is expected to present the results of chromatogram of the metal mixture separation, from the mixture separation of 3 metal-DBDTC complexes

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and the mixture separation of 4 metal-DBDTC complexes.

The mixture of 3 metal-DBDTC complexes

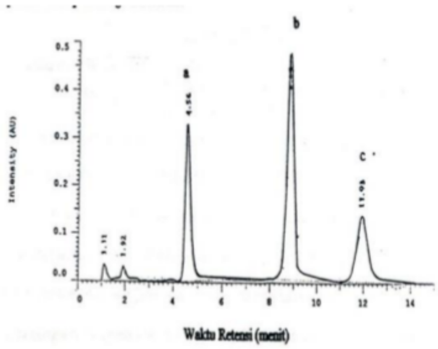

Figure 3. The chromatogram of metal complex (a) Co (II) DBDTC; (b) Cu (II) DBDTC; (c) Hg (II) DBDTC with DBDTC ligand concentration of 0.01 M. λ of UV detector at 256 nm, the flow rate of 0.75 ml/min, methanol:water (70:30)

Figure 3 showed the chromatogram for a mixture of three metal- DBDTC complexes that is the metal Co (II) DBDTC, Cu (II) DBDTC, and Hg (II) DBDTC complex. The HPLC conditions above were seen that there was a good separation of the three complexes.

The mixture of 4 metal-DBDTC complexes

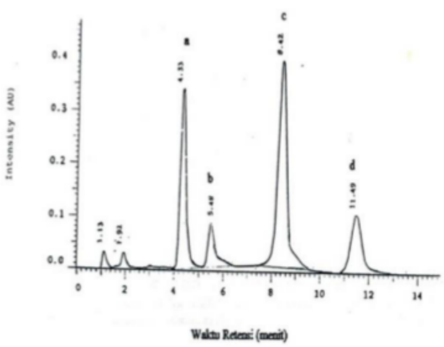

Figure 4. The chromatogram of metal complex (a) Co (II) DBDTC; (b) Ni (II) DBDTC; (c) Cu (II) DBDTC; (d) Hg (II) DBDTC with DBDTC ligand concentration of 0.01 M. λ of UV detector at 256 nm, the flow rate of 0.75 ml/min, methanol:water (70: 30).

Figure 4. showed the chromatogram of the mixture of four metal DBDTC complexes that is the metal Co (II) DBDTC, Ni (II) DBDTC, Cu (II) DBDTC, and Hg (II) DBDTC complex. in the HPLC conditions above was seen that there was a good separation for the four metal-DBDTC complexes, with the value of $t_{R\ Co}$ = 4,33; $t_{R\ Ni}$ = 5,42; $t_{R\ Cu}$ = 8,42; and $t_{R\ Hg}$ = 11.49. The shift of t_R became smaller (more polar), it was possible that the addition of Ni (II) led the mixture of metal DBDTC complex becoming more polar.

Conclusions

The inquiry process could produce the separation condition of Co, Ni, Cu, and Hg by HPLC reversed-phase using C_{18} column, with eluent methanol: water (70:30) and flow rate 0.75 ml/min, UV detector mounted at λ 256 nm, a mixture of five complex metals DBDTC could be separated well. According to the calculation of column capacity factor and resolution column in the ligand-n-butilditiokarbamat is selective enough to separate five complex metals DBDTC with reversed phase, C_{18} column.

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