International Association of Scientific Innovation and Research (IASIR)
(An Association Unifying the Sciences, Engineering, and Applied Research)

ISSN (Print): 2279-0047
ISSN (Online): 2279-0055

International Journal of Emerging Technologies in Computational and Applied Sciences(IJETCAS)

www.iasir.net

Measurement of Uric Acid Level In Vivo by Reverse Iontophoresis

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Abstract: *The routine method of the blood sampling for measuring uric acid level is achieved by using a syringe which is an invasive method. Reverse iontophoresis is an alternative non-invasive method for sampling of uric acid in the blood. The aim of the present work was to determine the effectiveness of reverse iontophoresis method on measurement of uric acid level in rat. The research was conducted by using an in vivo modified diffusion cell and wistar strain male rats. The reverse iontophoresis method was used 0,5 mA/cm2 current density with a modified iontophoresis device. The average uric acid levels after diffusion testing for 6 hours was 1.73 ppm, while in the blood were 63.8 ppm. The coefficient correlation between the levels of uric acid in the blood and the levels of uric acid in the receptor fluid was 0.5215. In the previous study, the in vitro study indicated that uric acid had been successfully extracted through the abdominal skin of rat to a collection solution of modified diffusion cell as much as 7.35 ppm. In conclusion, in vitro and in vivo studies showed the possibility to describe the levels of uric acid from interstitial fluid with reverse iotophoresis method.*

Keywords:*uric acid, reverse iontophoresis, in vivo, invasive, rats*

I. Introduction

Gout is the most common inflammatory arthritis in the elderly population.[1]Closely associated with a metabolic disorder of uric acid in the body that can cause abnormal uric acid levels in the blood. The condition is known as hyperuricemia. It may exist for several years to decades before the first symptoms of gout attacks appear, therefore, the disease associated and correlated with aging.[2]

In general, method to determine blood uric acid level is achieved by blood sampling. However, this method is invasive, painful, and inconvenient. Reverse iontophoresis can be used as alternative method that is non-invasive to determine the blood uric acid level.[3] Reverse iontophoresis refers to the passage of a low level of current through the skin to promote the transport of both charged and neutral molecules.[4] The main mechanisms that contribute to Reverse iontophoresis are the electromigration of charged species to the electrode of opposite polarity, electroosmosis of neutral molecules to the cathode or anode, or a combination of both.[5]

The purpose of this study was to determine the effectiveness of reverse iontophoresis method on determination of uric acid level in vivo in rat.

II. Materials and Methods:

A. *Materials*

Ammonium acetate (Merck), aquabidest (IPHA), uric acid (Sigma Aldrich), phosphoric acid 85% (Merck), acetonitrile pro HPLC (JT beaker), Disodium Edetas (Merck), pure Ag wire (PT. Antam, Tbk), Platinum/Pt wire (PT. Antam, Tbk), KCl (Merck), methanol pro HPLC (JTbeaker), sodium Hydroxide (Merck), blood plasma (PMI Bandung), monobasic sodium phosphate (Merck), and wistar strain male rats (PAU ITB).

B. *Equipment*

A modified diffusion cell, modified Iontophoresis devices, 99.95% pure silver wire 1 mm diameter, 99.95% pure platinum wire, pH-metre (Metroohm Type 774), spectrophotometer UV-Vis (Specord Analytic Jena 200-222U179) membrane millipore 0,45 µm, vortex; centrifugation apparatus (Hettich); HPLC instrument Shimadzu (Liquid Chromatograph LC-6A, Auto Injector SIL-9A, UV Spectrophotometric Detector SPD-6A, Chromatopac CR 501) ; Waters Spherisorb S5 C18 (250x4.6 mm SS, Waters USA); Sonicator Branson 5200

C. *Preparation of modified diffusion cell*

The diffusion cell for in vivo studies was adopted and modified from Chih-Kuei et al(2010) as described in figure 1.[3]

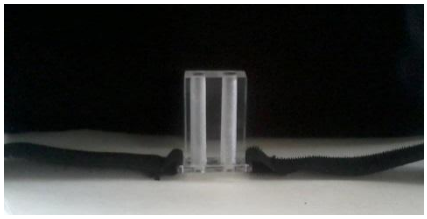

Figure 1. In vivo diffusion cell

D. Preparation of rats

10 wistar strain male rats aged 12-16 weeks, 180-280 g weight was used in the in vivo study. It was induced by uric acid with a dose of 1 g / kg and potassium oxonate with a dose of 200 mg / kg.

E. Preparation of Ag/AgCl electrode and modified iontophoresis device

The preparation method of Ag/AgCl electrode and modified iontophoresis (figure 2) were adopted from Wathoni et al study.[6,7]

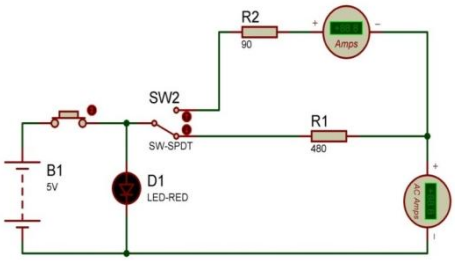

Figure 2. The electrical circuit of reverse iontophoresis device

F. Optimization of Condition Analysis Uric Acid in Blood Plasma

Optimization was done by varying Amonium Acetate (0,01 M, pH 5,0) 100 % v/v : Amonium Acetate (0,01 M, pH 5,0) : Acetonitril (98: 2 v/v) as the mobile phase.

G. Validation of analytical methods in Blood Plasma

Validation of analytical methods consist of determination of the parameters: linearity; accuracy; precision; suitability test.

H. Meausuring uric acid levels in vivo using modified iontophoresis device:

The series of electrode Ag / AgCl, modified iontophoresis devices and in vivo modified diffusion cells were prepared and applied to the abdominal skin of rat with a constant current density of 0,55 mA/cm2. Each chamber of the modified diffusion cell electrode was filled with the receptor phosphate buffer pH 7.40. The samples were taken as much as 1 ml from receptor fluid after diffusion testing for 6 hours and were analyzed by HPLC. The results were compared with the data of plasma uric acid in rats blood with syringe method.

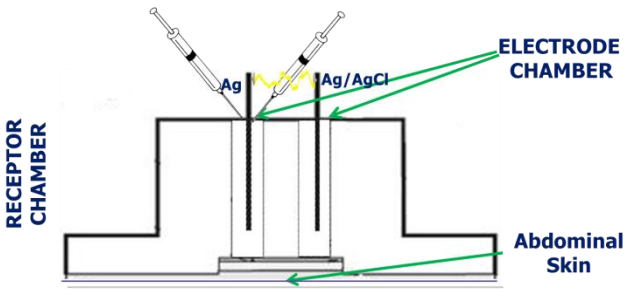

Figure 3. In Vivo Scheme

I. Statistical analysis used:

A linear regression test was used to determine the relationship between the subject's blood uric acid level and uric acid concentrations in collection solutions, with standard deviation for each data.

III. Results

In this study, acetate buffer (pH 5) and acetonitrile with the composition 98:2 v/v showed the best chromatogram result with a flow rate of 0.5 ml/min. Uric acid retention time resulting from the optimization of the analysis was on 6.183 minutes. Chromatogram resultcan be seen in figure 7.

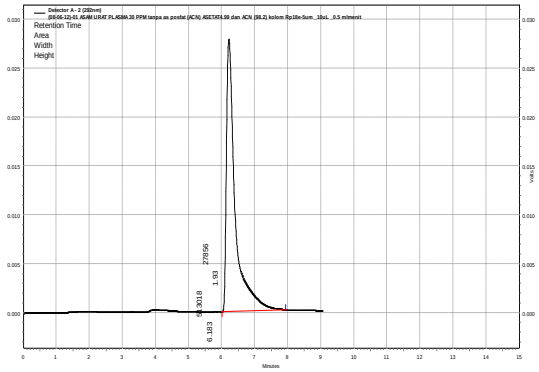

Figure 7. Chromatogram ofuric acid

Results of calibration curve for the validation of analytical methods of uric acid in the blood plasma was in the figure 8.

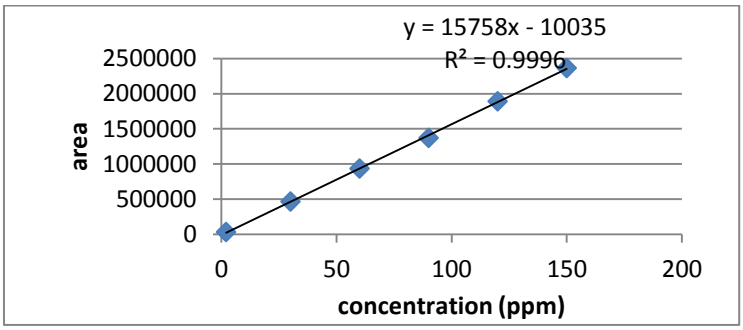

Figure 8. Result of linearity determination

The results of linearity determination obtained a linear relationship with a correlation coefficient (r) = 0.999 and the regression equation y = 15758x-10035.

Table 1. Result of accuracy determination

Sample (ppm)	AUC	Conc. (ppm)	% recovery	
30	462582,5	29,99223	99,97409	
	471201	30,53915	101,7972	**101,2111**
	471507	30,55857	101,8619	
90	1344267,5	85,94381	95,49312	
	1353861,5	86,55264	96,1696	**95,91884**
	1352786,5	86,48442	96,0938	
120	1889775,5	120,5617	100,468	
	1885075,5	120,2634	100,2195	**99,29927**
	1828172,5	116,6523	97,21028	
			%	**98,80974**

From the table 1, it showed that the percent of recovery obtained from the 3 variations of concentration qualified with FDA requirement in the range 80-120% which the percent of recovery average was 98.80974%.

FDA precision criteria will be accepted if the method give a coefficient of variation of 2% or <2%, and <10% for biological system. The average RSD (relative standard deviation) value in this study was 1.09050 %.

Based on the analysis, the system was in compliance with the suitability test because the value of % coefficient of variation for the retention time was 0.63067% and for the chromatogram area was 1.23114 %.

The blood of the rats were taken atsixth hour after induced by uric acid and potassium oxonate. Blood plasma were extracted using acetonitrile. This method was chosen because the extraction method is relatively easy to perform and produce a good separation of analytes. The result of uric acid level with syringe sampling method was 63,8±27.95 ppm

Table 2. Result of precision determination

Sample (ppm)	AUC	Conc. (ppm)	RSD
30	462582,5	29,99223	
	471201	30,53915	1,058915
	471507	30,55857	
90	1344267,5	85,94381	
	1353861,5	86,55264	0,386398
	1352786,5	86,48442	
120	1889775,5	120,5617	
	1885075,5	120,2634	1,82618
	1828172,5	116,6523	
		Average	1,09050

Table 3. The results of uric acid levels with syringe sampling method after 6 hours (n=5)

Rats	**Levels of Uric Acid in the blood (ppm)**
1	90
2	44
3	33
4	56
5	96
Average	**63,8**
standard Deviation	**27.95**

The result of the uric acid level with reverse Iontophoresis sampling method in the receptor fluid after diffusion for 6 hours was 1.73±1.41 ppm.

Table 4. The results of the uric acid levels with reverse Iontophoresis sampling method in the receptor fluid after diffusion for 6 hours (n=5)

Rats	Uric Acid Levels in the receptor fluid (ppm)
1	0.75
2	1.16
3	3.00
4	3.45
5	0.27
Average	**1.73**
Standard deviation	**1.41**

The levels of uric acid by reverse iontophoresis methodwere comparedwith syringe methodand gave the linier regression at 0.5215.

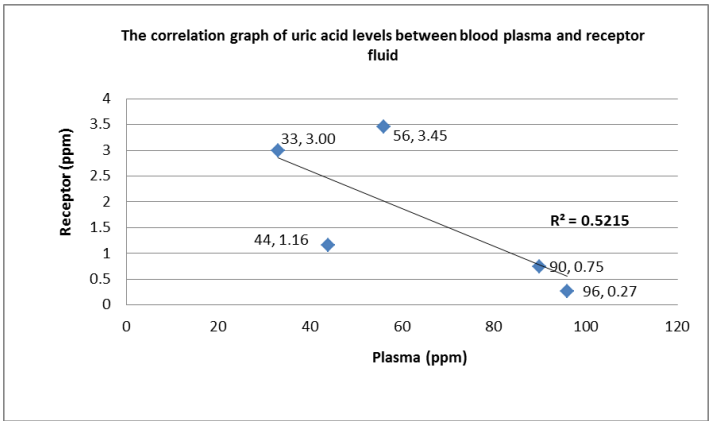

Figure 9. Correlation graph between the concentration of uric acid in the blood with syringe and the receptor fluid after application with reverse iontophoresis for 6 hours

IV. Discussion:

Reverse Iontophoresis is the blood sampling method by applying a low electrical current to pass through the skin and it can carry out charged or uncharged (neutral) molecules. A low electric current is applied to the skin to pull or extract the analytes from the blood. The mechanism of reverse iontophoresis is a combination of electromigration and electroosmosis process in the body.[4,8]

At pH below the pKa of uric acid will form nonionized molecules. The use of buffer with a pH below the pKa of uric acid as the mobile phase and the addition of acid made to maintain uric acid in the form of nonionized molecules during the process of analysis using HPLC. The form of uric acid in the body's normal pH (pH 7.4) is ionic form. Therefore, uric ions can be extracted out through the skin with the help of a constant current strength. Electromigration mechanism in the reverse iontophoresis method will make the negatively charged of uric ions going towards the positively charged electrode, and it will be extracted in the anode chamber in the diffusion cell apparatus. Electroosmosis mechanism influence the molecules of uric acid in the body diffuse towards the cathode. The diffusion of uric acid using reverse iontophoresis extraction will produce uric acid in the anode and cathode chamber diffusion cells and it can be calculated.

The low extraction values obtained in reverse iontophoresis study could be potentially caused by several factors such as weight and body surface area. It also can be caused due to the small ion content in the rat's body. The electrical conductivity is reduced through the skin. Uric ions were formed in small amount probably because uric acid are not ionized well in the rat's body. The uric acid is a weak acid with pKa1 5.75 and pKa2 10.3.[9]The large standard deviation value can be tolerated because it is generally associated with testing of living things (animals) tend to be large due to differences in metabolic factors of each animal experiments.

The result of correlation between the concentration of uric acid in the blood with syringe and the receptor fluid after application with reverse iontophoresis indicated that many factors can influence the correlation coefficient (r2), such as sample size, type of the variable to be studied, and others. Better correlation without removal of outliers from the linear regression line can be obtained if the sample size was large enough so that the variation might be as low as the data obtained from randomized data. In addition, various types of data can be included in the correlation analysis, this will provide a wider range of data to be analyzed and the correlation will bemore reliable. From the chart above, the correlation between uric acid levels was 0.5215. The correlation between the levels of uric acid in the blood and the ratio of uric acid levels in the receptor fluid is still not too high. In the previous study, The in vitro study indicated that uric acid had been successfully extracted through the abdominal skin of rat to a collection solution of modified diffusion cell as much as 7.35 ppm.[6] In conclusion, in vitro and in vivo studies showed the possibility to describe the levels of uric acid from interstitial fluid with reverse iotophoresis method.

V. References

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V. Acknowledgments

Funded in part by the HibahBersaing DIKTI (the Indonesian Ministry of Education and Culture). Thanks CyntiaFebrianti and Santo Gultom for theirs helpful contributions.