DEVELOPMENT AND VALIDATION ZIC HILIC COULOMN FOR SIMULTANEOUS DETERMINATION OF FUROSEMIDE AND INDAPAMIDE AS DOPING

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Abstract

In sports, doping abuse done by athletes has been done since the 18th century. Many of the drugs used for doping and its chemical properties vary. Based on a list issued by the World Anti Doping Agency, one of the doping categories is diuretic. Then the most appropriate testing method is needed to separation furosemide and indapamide. This study aims to see the development and validation of the separation furosemide and indapamide as doping compounds with High Performance Liquid using the Zic - HILIC column.

This study used High Performance Liquid Chromatography of Shidmazu LC 20 AD brand with iso-fed pump and SPD 20A UV-Vis detector. The column used is Zic - HILIC (150 x 4.6 mm; 5 μ m). This research is carried out on the optimization of conditions including mobile phase velocity, mobile phase pH, buffer of mobile phase, and buffer concentration. Selection of selected conditions see the price of $R > 1.5$ and $N >>>$ or HETP $<<<$ Optimized wavelength results 276 nm. The separation conditions of the furosemide and indapamide mixtures by HPLC using Zic-HILIC columns (150 x 4.6 mm, 5 μ m) are Acetonitrile: Ammonium Acetate buffer (5 mM, pH 5) (95: 5) with a velocity of 0.5 ml / minute. The selected condition gives the given resolution value > 1.5 and the N price is the greatest. The method was than validated for Selectivity, linearity, accuracy, precision, limits of detection and limits of quantification. The calibration curve of furosemide and indapamide were linear with a regression coefficient $R > 0.999$.

Keyword : Zic Hilic Coulomn, Furosemide, Indapamide

Introduction

Sports is a place where there is a process of interaction between humans and contains ethical values that are shown, tested and learned from each other. The value involved in sports does not only include body or intellectual involvement but also humanity as a whole, such as fair play, teamwork, and sportsmanship. Since 1967, have been deaths among cyclists, soccer players and boxers due to the use of "wake amine" or "vasodilatator" and contestants who died of heroin poisoning. There is no sport that is free from doping to this day. (Müller R.T, 2010) Based on the list issued by

the World Anti-Doping Agency, there are 9 doping categories, one of which category is a diuretic. (Anonim, 2014)

Then the most suitable testing method is needed to analyze diuretic drugs. The analytical method that is often used in doping analysis is gas chromatography, liquid chromatography, and electrophoresis. The problem is analyzing the drug for hydrophilic compounds (Park, J., et al., 1989; Hatton, C. K, 2007; Kolmonen .M, 2011). Furosemide and Indampide are a class of diuretic drugs that can be used as doping. But Furosemide and Indapmide including hydrophilic drug

compounds are difficult to separate and analyze.

Until now, widely known separation models at HPLC include normal (NP), reverse phase (RP), ion exchange, exclusive size, chiral separation and affinity models. But for the separation of polar compounds it becomes a problem (Ikegami et al, 2006). One solution to the problem for hydrophilic compounds has been done using the HILIC column (Ikegami et al, 2006).

Methods

Apparatus

HPLC Type Agilent 1100 Series Low Presre Gradient, Column: ZIC - HILIC 100 mm x 2.1 mm x 5 μ m, Photo Diode Array (PDA) detector, Hewlett Packard V - Vis Spectrophotometer HP) 8452 A, Analytical balance Mettler Toledo 37600, , Socorex Calibra 822 made Switzerland, nylon membrane filter 0.2 μ m, 13 mm Whatmann and filters 0.2 μ m, 47 mm and glassware

Reagent

Furosemide (Sigma), Indapamide (Sigma), Ammonium Acetate (Merck), Sodium Acetate (Merck), Acetonitrile for HPLC, Methanol for HPLC, Aqua for HPLC

Figure 1. The structure of Furosemide (a) and Indapamide (b)

Sample Preparation

Thoroughly weighed standard Furosemide and Indapamide with concentration 1.0 mg / ml in Acetonitrile: Water (90: 10)

Optimum wavelength

The standard solution was prepared from standard solution of Furosemide and Indapamide each 100 ppm, filtered by Whatman membrane filter of 0.2 μ m pore diameter and then injected to the HPLC as much as 20 μ l. Spectra is seen using Diode

Array detector at HPLC. Observation of spectra at wavelength 200 - 400 nm.

Optimization HPLC

Optimization of HPLC conditions with some effect of the condition in the analysis. Conditions made include: mobile phase composition, speed of mobile phase, buffer concentration, buffer type, organic solvent and pH. The standard mixed solution was injected as much as 20 μ l using a composition which was varied based on the effect of the condition in the analysis.

Result and Discussion

Effect of concentration phase concentration variation

Table 1. Effect of concentration phase concentration variation

Acetonitril : Dapar Amonium Asetat	Compound	N	R
95 : 5	Furosemide	3983.422	2.64
	Indapamide	7440.957	
90 : 10	Furosemide	1029.453	1.43
	Indapamide	2461.573	
85 : 15	Furosemide	913.105	0.54
	Indapamide	4106.128	

The mobile phase composition of Acetonitrile: Ammonium Acetate buffer with a selected between (95: 5), (90:10) and (85:

15) is (95: 5) . The given resolution is 2.64 (> 1.5) and the largest N.

Effect of pH variation of buffer

Table 2. Effect of pH Ammonium Acetate

pH Amonium Acetate	Compound	N	R
4,0	Furosemide	-	-
	Indapamide	-	
5,0	Furosemide	3983.22	2.64
	Indapamide	7440.957	
6,0	Furosemide	3107,00	1.76

The pH variation of the ammonium acetate buffer in the mobile phase comparison selected between pH 4, pH 5

and pH 6 is pH 5, this is because the resulting resolution is 2.64 (> 1, 5) and the largest N.

Effect of buffer concentration

Table 3. Effect of Ammonium Acetate Concentration

pH Ammonium Acetate	Compound	N	R
5,0 mM	Furosemide	3983.422	2.64
	Indapamide	7440.957	
10,0 mM	Furosemide	2503.363	2.27
	Indapamide	5323.411	
20,0 mM	Furosemide	1898.422	1.84
	Indapamide	4044.957	

Variations of concentration of Ammonium Acetate buffer (pH 5) selected between 5 mM, 10 mM and 20 mM were 5 mM. The

resulting resolution is 2.64 and the value N is the greatest.

Effect of flow rate

Table 4. Effect of flow rate

Flow rate	Compound	N	R
1,0 ml / minute	Furosemide	1848.230	1.24
	Indapamide	4182.315	
0,75 ml /minute	Furosemide	2427.516	1.45
	Indapamide	3915.660	
0,5 ml/minute	Furosemide	3234.213	1,99

Variations in flow rate velocity between 1ml / min. 0.75 ml / min and 0.5 ml / min selected is 0.5 ml / min. The given resolution

is> 1.5 and the N price is the greatest of the three speeds varied.

Figure 2. Effect of Ammonium Acetate Concentration Variation

Methode Validation

After the found of conditions were optimized separated Furosemide and

Indapmide, The method was validated as ICH guidelines such as linearity, accuracy, precision, LOD and LOQ

Liniearitas

Figure 3. Linearity of Furosemid and Indapamide

Table 5. Liniear Regersiaon data of Furosemid and Indapamide

Validation Parameter	Furosemide	Indampide
Liniearity Range	2,0 - 25	5 – 25
Correlation coefficient	0.9997	0.9988
LOD	0,6025	0,8694
LOQ	1,8258	2,6346

Corelation coefficientfor Furosemide and Indapamide should be equal or greater than 0.999 (R calc. > R table; Vxo eligibility no more than 5 %)

Accuracy and Precision

	Accuracy			Precision (CV)
	80 %	100%	120 %	
Furosemide	100.07% $\pm$ 0.63	99.68% $\pm$ 0.20	99.73 % $\pm$ 0.65	1.99 %
Indampide	99.72% $\pm$ 0.50	100.26 % $\pm$ 0.78	99.70 % $\pm$ 0.09	1.84 %

Accuracy and precision of furosemide and indapamide accepted to requirement (acceptance criteria 80 – 110 % for Accuracy and precision CV is less than 2 %)

Conclusion

The separation conditions of the furosemide and indapamide mixtures by HPLC using Zic-HILIC columns (150 x 4.6 mm, 5 μ m) are Acetonitrile: Ammonium Acetate buffer (5 mM, pH 5) (90: 10) with a velocity of 0.5 ml / minute.

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