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Analysis of Salicylic Acid in Biological Fluids (Serosal) Using Uv-Vis Spectrophotometry

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ABSTRACT

Pharmacokinetics is an aspect of pharmacology that covers the fate of drugs in the body, namely absorption, distribution, metabolism, and excretion (ADME). Overall, bioavailability shows the kinetics and comparison of the levels of active substances that reach the bloodstream to the amount of drug given. Method validation is carried out to determine that the analytical method used is accurate, specific, reproducible, and resistant to the analyte to be analyzed. An analysis model must be validated to verify that its performance parameters are sufficient to overcome the analysis problem. The research method used is included in observational experimental research. Observational experimental research is research conducted by analyzing a compound and testing it. The results of this study obtained a standard curve value of Y = 0.006x + 3.401, r2 = 0.334 with λmax 329 nm. Recovery (% recovery) in the study, the average recovery value of the concentration was 200µg/mL (118.8%); 150 µg/mL (72.1%); 100 µg/mL (131.5%). Systemic error of series concentration 200 µg/mL (-18.8%); 150 µg/mL (278%); 100 µg/mL (-31.5%). Based on the study, validation testing of the method with further precision and accuracy is needed.

Keywords: Cimetidine, Diazepam, Effects, Metabolism, Mice

INTRODUCTION

Fase farmakokinetika berkaitan dengan masuknya zat aktif ke dalam tubuh. Ketersediaanhayati adalah sifat suatu obat yang diberikan pada sistem biologis didalamtubuh secara utuh. Secara keseluruhan ketersediaan hayati menunjukkan kinetika danperbandingan kadar zat aktif yang mencapai peredaran darah terhadap jumlah obat yangdiberikan. Sedangkan untuk studi biofarmasi dendiri merupakan evaluasi karakteristikkuantitatif dan kinetik suatu obat tertentu dan pada organisme tertentu, selain itupenggunaan cuplikan in vitro berasal dari tubuh seperti saluran cerna (Syukri, 2002). Validasi metode dilakukan untuk menentukan bahwa metode analisis yang digunakanakurat, spesifik, reprodusibel, dan tahan pada analit yang akan dianalisis. Suatu modelanalisis harus divalidasi untuk melakukan verifikasi bahwa parameter-parameterkinerjanya cukup mampu untuk mengatasi problem analisis. Beberapa parameter yang digunakan adalah akurasi, presisi, dan spesivitas. Akurasi merupakan ketelitian metode analisis antara nilai terukur dengan nilai yangditerima baik nilai konvensi, nilai sebenarnya, atau nilai rujukan. Akurasi diukursebagai banyaknya analit yang diperoleh kembali pada suatu pengukuran denganmelakukan spiking pada suatu sampel. Pengukuran diperoleh dari pengumpulan datadari 9 kali penetapan kadar dengan 3 konsentrasi yang berbeda (3 konsentrasi dengan3 kali replikasi). Data harus dilaporkan sebagai presentasi perolehan kembali. Presisimenunjukkan keterulangan nilai yang diperoleh dengan metode yang sama baikdilakukan dengan kondisi yang sama maupun berbeda. Spesifitas adalah kemampuanuntuk mengukur analit yang dituju secara tepat dan spesifik dengan adanya komponen- komponen lain dalam matriks sampel seperti ketidakmurnian, produk degradasi dankomponen matriks. (Gandjar & Rohman, 2007). Terdapat banyak metode yang dapatdigunakan untuk mengetahui ketersediaan hayati suatu obat. Oleh karena

itu perludilakukan validasi terhadap metode yang digunakan untuk membuktikan bahwa setiapbahan, proses, prosedur, kegiatan, sistem, perlengkapan atau mekanisme yangdigunakan dalam produksi dan pengawasan mencapai hasil yang diinginkan. (Lachmanet al., 1994).

Biological samples are samples taken from body parts for analysis purposes. Biological samples that are generally used to determine drug compound levels in the body are blood plasma. Blood plasma is used as a biological sample in determining drug compound levels because the concentration of the drug will bind to its receptors and determine the magnitude of the pharmacological effects given by a drug, where these receptors are mostly found in tissue cells, so examining drug levels in the blood is the most accurate method for monitoring treatment/clinical pharmacokinetics (Rizalina et al., 2018). 3 Biological matrices are materials other than the analyte in biological samples. Biological matrices that are often used to analyze drug levels or other compounds are serum, urine, blood, and saliva. Biological matrices (especially blood) are obtained invasively through a vein (Venipuncture) (Supandi, 2015). Before preclinical tests and clinical pharmacology tests are carried out, an analysis method must first be validated. Validation of analytical methods is a process to prove that the performance of the analytical method has met the requirements that have been set in accordance with the desired use. (Validation Method for Analysis of Chemical Compounds of Drugs in Biological Samples (Blood Plasma).

Validation of analytical methods is a process that ensures accurate, specific, reproducible, and resistant analysis in the range of analytes to be analyzed in accordance with the desired use (Fauziah et al., 2017). Method validation according to the United States Pharmacopeia (USP) is carried out to ensure that the analytical method is accurate, specific, reproducible, and resistant to the range of analytes to be analyzed originating in biological matrices, such as blood, plasma, serum, or urine, can be trusted and can be repeated for the desired use (Fauziah et al., 2017). Several analysis parameters that must be considered in the validation of analytical methods are described and defined as well as how to determine them. The parameters include accuracy, precision, selectivity, linearity and range, limit of detection (LOD) and limit of detection (LOD). quantity (LOQ), method robustness, method strength (Nyoman et al., 2015). An analysis method can be used if it has been validated and adjusted to the laboratory conditions and available equipment, even though the method to be used has been published in an official journal or textbook (Uno et al., 2015). There are several method validations that are generally used in determining the levels ofdrug compounds in blood plasma, namely the TLC-densitometry method, High Performance Liquid Chromatography (HPLC) with UV detector, SPE-MIP, Ultra Performance Liquid Chromatography (UPLC) and UV-VIS Spectrophotometer. (Validation Method for Analysis of Chemical Compounds of Drugs in Biological Samples (Blood Plasma) 4.

METHODS

Tools used: UV spectrophotometry, centrifuge, volume pipette, flaskmeasuring/measurement, beaker glass and stirring rod, scalpel tube, micropipette, cuvette, filter paper. Materials used: Salicylic Acid, Serosal Solution (NaCl 0.9% b/v) and FeCl3.

Research Procedure Making a stock solution of 2000 ppm salicylic acid by weighing 0.04 grams of salicylic acid and then dissolving it in 20 ml of distilled water and filtering it. Making a serosal solution is by weighing 0.9 grams of NaCl in a beaker that has been tared, adding 100 ml of distilled water, the serosal solution is ready to use. Determination of Operating Time and max, namely Take 1 mL of salicylic acid measuring flask add 10 mL using serosal (200 µg/mL series) From the parent solution pipette 875 µL and add 10 mL with serosal solution (175 µg/mL series) Pipette 750 µL of the parent concentration ad 10 mL

of serosal solution (150 µg/mL series) Pipette 625 µL of the parent solution ad 10 mL of serosal solution (125µg/mL series) Pipette 500 µL of the parent solution ad 10 mL of serosal solution (100 µg/mL series) Add 1 mL of FeCl3 reagent to all concentration series Take the middle value, namely in the third concentration series to determine OT Insert the cuvette and measure the absorbance with a spectrophotometer at λmax and determine OT Determination of λmax carried out multilevel dilution at the five concentrations (200 µg/mL, 175 µg/mL, 150 µg/mL, 125 µg/mL, 100 µg/mL) Then insert the cuvette and measure the max wavelength in the absorbance range of 300-600 nm d. Determination of λmax Add 1 mL of FeCl3 reagent to all concentration series Take the middle value, namely in the third concentration series to determine OT Making a Standard Curve Series 1) Make a standard curve with 5 concentrations Concentration Series (µg/ml) Salicylic Acid (µl) Serosal (µl) 200 1,000 9,000 175 875 9,125 150 750 9,250 125 625 9,375 100 500 9,500 2) In the first concentration series, take 1000 µl of salicylic acid solution 3) Add serosal ad 10 mL□ series 1 4) Take steps from the concentration series to make the concentration series 2 and so on up to the 5th series by taking according to the calculations in the table 5) Add 1 mL of FeCl3 to each concentration series 6) Let stand for OT and enter blank (aquadest) then enter the previously prepared series 7) Measure the absorbance at lambda max using a spectrophotometer Determination of 3 series of High, Medium, and Low concentrations Make the highest concentration series 200 µl, medium 150 µl and low 100 µl with the same procedure as the standard curve determination procedure Then enter the blank then enter the prepared series in the cuvette and measure the absorbance at the maximum wavelength Do 2 replications.

RESULTS

Research ProcedureThe results of this study show the analysis of salicylic acid in biological fluids (serosal). The purpose of this study was to validate the analysis method using uv-vis spectrophotometry. The first step taken was to make a stock solution of salicylic acid 2000 ppm or µg/mL, then a serial dilution of graded concentrations was carried out to 200, 175, 150, 125, and 100 µg/mL. The material used was salicylic acid with a standard curve of $Y = 0.006x + 3.401$, $r\ 2 = 0.334$ with λmax 329 nm.

The results of the analysis of salicylic acid in biological fluids (serosal) aim to validate the analysis method using uv-vis spectrophotometry. Salicylic acid (AS) is one of the beta-hydroxy acids and is a compound that can be easily found in willow trees. Salicylic acid also has a unique ability to smooth and shine the skin as is commonly applied in cosmetic products. At present, the application of the use of cosmetic products has been widely circulated in the market and used by all levels of society. Its uses are diverse, such as skin care, beauty and even for the treatment of skin diseases / sensitive skin. In addition, according to studies conducted by dermatologists, salicylic acid is a common compound that is commonly used in various types of cosmetic and skin care products. Salicylic acid or known as beta-hydroxy acid (BHA), is a keratolytic compound that has the ability to loosen the outer layer. BHA also has an effect that can whiten the skin and can eliminate acne on the face. However, the use of salicylic acid compounds should also be limited, this is because this compound can have negative effects on the user's skin such as irritation, redness and itching. According to the Chinese Hygienic Standard (CHS) for cosmetics, the content of salicylic acid in cosmetic preparations must be below 2.0%, this content limit is the same as according to the Food and Drug Supervisory Agency of the Republic of Indonesia. Many methods have been developed in determining the levels of salicylic acid in cosmetic preparations such as capillary chromatography, high-performance liquid chromatography4, ion-exclusion chromatography, capillary electrophoresis and spectrophotometry. UV-Vis

spectrophotometry is considered one of the best analysis methods, this is because the analysis process is simple and this instrument is widely used in most quality control laboratories.

DISCUSSION

In this study, salicylic acid analysis was carried out using the UV-Vis spectrophotometry method. The first step taken is to make a stock solution of salicylic acid 2000 ppm or µg/mL, then a dilution of a series of graded concentrations is carried out to 200, 175, 150, 125, and 100 µg/mL. Analysis using a uv-vis spectrometer and the maximum wavelength is obtained (knowing how much the maximum wave absorption is) of 329 nm taken from the middle concentration series. While the operating time (the time the analyte is mixed with the solvent) is not carried out. The third step is to determine the standard curve, from the 5th readingconcentration series obtained Y = 0.006x + 3.401 with r 2 is 0.334. While in the high, medium and low concentration series with 2x replication of readings obtained Y = 0.008x + 3.009 with r 2 is 0.591. The linearity test is shown based on the acceptance criteria for the correlation coefficient is $r \geq 0.995$ (Agustina & Sujana, 2020). It was obtained from this study that a non-linear relationship was obtained between concentration and absorption. 10 Accuracy or precision value is a measure that indicates the degree of closeness of the analysis results to the actual analyte levels. Accuracy is often expressed as the percent recovery (% recovery) of a test against the addition of a known amount of analyte. This accuracy test is carried out to see the accuracy of the tool and analysis in making a solution concentration that matches the actual levels (Mulyati & Apriyani, 2017). Accuracy test with the parameter percent recovery (% recovery). Accuracy test with percent recovery parameters was carried out by measuring 3 analyte concentrations with high, medium, and low concentrations as many as 2 replications. The average recovery value of 200 µg/mL concentration (118.8%); 150 µg/mL (72.1%); 100 µg/mL (131.5%) was obtained. This percent recovery is unacceptable because it does not meet the accuracy requirements where the average range of percent recovery results is 80-110% (Agustina & Sujana, 2020). How to determine the systemic error value, The systematic error value indicates the precision or accuracy of the method used. The systematic error value should be <10% so that the results can be said to be precise or accurate. This error is constant and results in certain deviations from the average. The systemic error of the concentration series was obtained 200 µg/mL (-18.8%); 150 µg/mL (278%); 100 µg/mL (-31.5%). From the calculation results, there is no systematic error value.

CONCLUSION

From the measurement of salicylic acid in serosal biological fluids using a uv-vis spectrophotometer, a standard curve of a series of 5 concentrations was obtained, namely Y = 0.006x + 3.401 with r 2 of 0.334. While in the high, medium and low concentration series with 2x replication of readings obtained Y = 0.008x + 3.009 with r 2 of 0.591. From the results of the linearity of the correlation coefficient (r) showed a non- linear relationship. 2. In the accurate test with the parameter percent recovery (% recovery). The average recovery value of 200 µg / mL (118.8%); 150 µg / mL (72.1%); 100 µg / mL (131.5%) was obtained. This recovery percentage is unacceptable because it does not meet the accuracy requirements where the average range of recovery percentage results is 80-110% 3. In the calculation of systematic errors, the systemic errors of the concentration series were 200 µg/mL (-18.8%); 150 µg/mL (278%); 100 µg/mL (-31.5%). From the calculation results, there were no systematic error values.

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