InternationalResearchJournalofBiotechnology(ISSN:2141-5153)Vol10(1)
Available online@http://www.interesjournals.org/IRJOB

EXTENDED ABSTRACTS

The effect of anticoagulant types in analyzing levofloxacin in human plasma by high performance liquid chromatography-photodiode array

Yahdiana Harahap, Agus Al Imam Bahaudin and Harmita

Universities Indonesia, Indonesia

Email: yahdiana03@yahoo.com

ABSTRACT

Levofloxacin has low concentration in plasma, thus it requires sensitive and selective analysis method. Plasma drug analysis often uses many sorts of anticoagulant to get plasma as analytical matrix. Citrate, heparin, and ethylenediaminetetraacetic acid (EDTA) are anticoagulant commonly utilized in analyzing drug in human plasma. This study was focused on analyzing levofloxacin in human plasma with three sorts of anticoagulants. The analysis was performed using High Performance Liquid Chromatography (HPLC) – photodiode array with Column C18 SunfireTM (250 x 4.6 mm), 5 µm; temperature of 45oC, mobile phase consist of 0.5% triethylamine pH 3.0 -acetonitrile (88:12 v/v); flow of 1.25 mL/minute, and ciprofloxacin HCl as internal standard. The method was linear at concentration range of fifty .0 – 10.000.0 ng/mL with r>0.9994. Accuracy and precision for citrate, heparin, and EDTA plasma fulfilled the acceptance criteria of both intra-day and inter-day. There was no significant difference for stability and recovery of levofloxacin in citrate, heparin, and EDTA plasma (p>0.05; ANOVA), but it showed significant difference for peak area ratio (p>0.05), between citrate-EDTA plasma and heparin-EDTA plasma for low concentration and between citrate-heparin plasma and citrate-EDTA plasma for mid and high concentration. On blank chromatogram EDTA plasma, there was interference on retention time of but 8 minutes, while on citrate and heparin plasma there was no interference. The method can be applied for bioequivalence study using the three anticoagulants that are equally good.

Clopidogrel is one of the slowest-onset action prodrugs (Brunton, Lazo, and Parker, 2006) and is the drug of choice for antiplatelet agents . Clopidogrel is also used as secondary prevention of cerebral and cerebrovascular infarction in patients who do not tolerate acetylsalicylic acid or experience new attacks while using acetylsalicylic acid . Clopidogrel is adenosine diphosphate receptor antagonist and irreversibly inhibits platelet function . The maximum concentration of clopidogrel in plasma is reportedly very low, that is, 7921.49±3921.39 pg/mL . Thus, developing accurate analytical methods for clopidogrel are important, particularly for the examination and monitoring of clopidogrel levels in plasma, which is that the commonest biological matrix for the analysis of drugs in the body. Plasma is obtained from blood samples with the use of anticoagulants. Thus, an important factor when obtaining plasma is the type of anticoagulant used and studies often use different anticoagulants, making comparisons between analytical methods difficult . The ethylenediaminetetraacetic acid (EDTA), heparin, and citrate are anticoagulants often used in drug

The anticoagulants themselves are generally present in the blood collection tube at a concentration sufficient to inhibit blood clotting, as the use of excess concentrations may lead to undesirable effects during bioanalysis [7]. However, the use of anticoagulants can lead to errors during the analysis of certain drugs . Thus, selecting the appropriate anticoagulant during drug analysis serves to provide many benefits, including minimizing interferences and improving the stability of the drug and/or its metabolites . Indeed, differences between anticoagulants have been known to affect the measurement of small molecules , metabolic profiles [6,10], and clinical parameters of the drug being analyzed [11-13]. In addition, a number of the ions (e.g., Na + or K +) present within the plasma anticoagulant like Na-citrate and K-EDTA may cause ion suppression or ion enhancement of the drug and its metabolites, which can affect analysis . Moreover, for plasma drug analysis, a selective and sensitive bioanalysis method is required because the plasma level of clopidogrel is extremely low . Ultra-highperformance liquid chromatography tandem-mass spectrometry (UHPLC-MS/MS) with electrospray ionization (ESI) is a specific and sensitive method of analysis that has become the standard for the measurement of drugs, metabolites and endogenous compounds in biological matrices .

This study was conducted to evaluate the commonly used anticoagulant types, namely, citrate, heparin, and EDTA, on parameters such as stability, recovery, matrix effect, and area

This work is partly presented at 10th International Conference on Applied Microbiology and Microbial Biotechnology, October 15-16, 2015 Ottawa, Canada

response of the analyte.

The internal standard used irbesartan because it has similar physicochemical properties to clopidogrel, particularly its acidity and solubility; thus, it can be detected and eluted using the same analytical method. The analytical conditions resulted in a good separation between the analyte and the standard with clopidogrel retention of 2.68 min and an irbesartan retention of 1.16 min. A relatively short run time of 4 min was also considered a good achievement of this method. Liquid-liquid extraction methods can produce larger analytical areas . In this study, the liquid-liquid extraction method was optimized. Liquid-liquid extraction is best used for analysis using HPLC-MS/MS because it does not damage the column when compared with the protein precipitation method. In addition, the resulting area response is greater due to the drying process, and fewer additional solvents (concentration process) are used. The optimization of the liquid-liquid extraction process conducted in this study was aimed to obtain an efficient and rapid extraction step so as to minimize contamination. However, during liquid-liquid extraction, an emulsion can sometimes form during processing, so the process needs to be repeated. Thus, a full validation was performed on plasma citrate .

The analytical method met the wants supported EMEA guidelines from 2011 for all three sorts of plasma (citrate, heparin, and EDTA). In addition, clopidogrel analysis in plasma citrate or heparin provides better results than in plasma EDTA.

Keywords: Citrate, Clopidogrel, Ethylenediaminetetraacetic acid, Heparin, Liquid chromatography tandem-mass spectrometry.

This work is partly presented at 10th International Conference on Applied Microbiology and Microbial Biotechnology, October 15-16, 2015 Ottawa, Canada