METHOD OF PHARMACEUTICAL ANALYSIS OF VALINE AND GLUTAMIC ACID IDENTIFICATION BASED ON THEIR FLUORESCENCE ABILITIES

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Abstract

L-Valine one of the compounds of parenterally infused feeding drugs, produced by the number of the countries (Aminoven , Germany; Highmix, Russia; etc.). It might be quantitatively estimated by means of Reverse Phase High Performance Liquid Chromatography and generation of isothiocyanate derivates of amino acids, which are time consuming and complicated methods. Another amino acid, glutamic acid, is applicable for the medicine as a compound improving cerebral blood flow and produced by the Sweden, Germany and Russia (Kabiven Central, Infesol 100, Eltacine).

One of the main reactions of glutamic acid identification is based on its green fluorescence abilities after interaction with the resorcinol. In accordance with the A.P. Arzamascev (Analysis of Pharmaceutical Chemistry) the reaction of green fluorescence is specific for glutamic acid. However, after comparison with the other amino acids we have found out that valine might be identified by the utility of the same method. Moreover, after utility of the equal quantity of the valine and glutamic acid, we concluded that green fluorescence of valine is more pronounced that glutamic acid in water environment , but not in alcohol, and the measurement as well as comparison of the fluorescence of these two compounds might serve as a new method for identification of valine as well as glutamic acid in a fast expressway in drugs. In this work, we introduce the new fast method of valine's identification.

Key words: glutamic acid, new method, valine, fluorescence, identification, resorcinol

Introduction

L-Valine one of the compounds of parenterally infused feeding drugs, produced by the number of the countries (Aminoven, Germany; Highmix,

Russia; etc.). It might be quantitatively estimated by means of Reverse Phase High Performance Liquid Chromatography (RP- HPLC) and generation of isothiocyanate derivates of amino acids, ion-exchange chromatography, NMR, which are time consuming and complicated methods[1,2]. Another amino acid, glutamic acid, is applicable for the medicine as a compound improving cerebral blood flow and produced by the Sweden, Germany and Russia (Kabiven Central, Infesol 100, Eltacine).

Adolf von Baeyer[3] for the first time in 1872 has reported about the formation of the compound, which was the phenol-based dye. It was forming from the mixture of concentrated sulfuric acid, benzaldehyde and resorcinol. Further, in 1940 Niederl and Vogel[4] studied the products obtained from the reaction between aliphatic aldehydes and resorcinol. They proposed the cyclic tetrameric structure (R1=aliphatic, R2'=H) analogous to cyclic tetrarneric structures frequently encountered in nature, e.g, porphyrins. This structure was finally proved in 1968 by Erdtman and coworkers by a single crystal X-ray analysis[5].

The concept that resorcinarenes can be used as templates for the synthesis of large macrocycles was introduced by Xuehe Li [6].

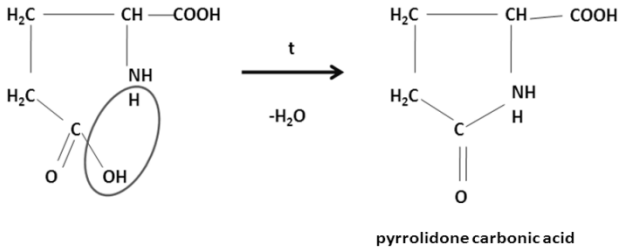
One of the main reactions of glutamic acid identification is based on its green fluorescence abilities after interaction with the resorcinol. In accordance with the A.P. Arzamascev (Analysis of Pharmaceutical Chemistry) the reaction of green fluorescence is specific for glutamic acid. However, after comparison with the other amino acids we have found out that valine might be identified by the utility of the same method. Moreover, after utility of the equal quantity of the valine and glutamic acid, we concluded that green fluorescence of valine is more pronounced that glutamic acid in water environment, but not in alcohol, and the measurement as well as comparison of the fluorescence of these two compounds might serve as a new method for identification of valine as well as glutamic acid in a fast expressway in drugs.

In this work, we introduce the new fast method of valines' identification by the utility of Fluorometer.

Materials and Methods

1. **Identification of glutamic acid and valine via their fluorescence abilities.** Glutamic acid (0.002 g) was mixed with the resorcinol (0.002 g) and 5 drops of concentrated sulfuric acid. The mixture was heated until the visualization of the greenish brown coloring. Further, the sample was cooled by the addition of the 5 ml water and 5 ml of 28% of the ammonium solution. The mixture obtained

redish-violet coloring with the green fluorescence. The same procedure was performed for valine. It was utilized Fluorometer from Perkin Elmer for the measurement of the intensity of the fluorescence. During the first step of reaction it is formed pyrrolidone carbonic acid from glutamic acid (**Scheme 1**), which further obtains more branched structure after the reaction with the Resorcinol [7].



Scheme 1. Formation of the first product of the condensation of the Glutamic acid.

2. **Statistical analysis.** It was used t-student test as well as ONE-WAY-ANOVA for calculation of the significance of the results. It is presented SEM on the graphs. The results were considered statistically significance when $p < 0.05$.

Results and discussion

1. Visualization as well as measurement of the glutamic acid's and valine's fluorescence intensity in the water solution.

After visual appreciation of the prevalence of valine's fluorescence intensity in comparison with the glutamic acid (**Fig.1. A., B**.) we have preformed series of the experiments to find out the optimal excitation as well as emission wavelengths. At the wavelengths λ_{Ex} =495 nm, λ_{Em}=515 nm, the intensity of the fluorescence for valine and glutamic acid in comparison with the Control was maximal. After several steps of dissolutions of the primer solution of the aminoacids we have noticed that the Fluorescence graph of the glutamic acid (solution used had $2x10^{-5}$, $2x10^{-6}$, $2x10^{-7}$, $2x10^{-8}$ mg/ml concentrations) possessed with the bell-like shape graph (**Fig 1. C, D**), where as the valine's graph was linear ($2x10^{-5}$, $2x10^{-6}$, $8x10^{-7}$, $2x10^{-8}$ mg/ml concentration). This finding evidenced about more pronounced valine's fluorescence in comparison with the glutamic acid.

2. Estimation of fluorescence intensity for valine and glutamic acid in water and alcohol environments.

The stock solutions for these experiments had concentrations equal to 0.0002 mg/ml of water or alcohol. These stock solutions were dissolved for 10x and 100x and the intensity of fluorescence for these solutions was measured (**Fig.2. A,B**). In both types of the environments, decrease of the solution's concentrations increases the intensity of the fluorescence. From Fig.2.A. it is clear that in the water solution dissolution for 100x increases the intensity of fluorescence of valine and not glutamic acid, whereas the 100x dissolution initiates the increase of the glutamic acid's fluorescence intensity.

Similar test system based on the measurement of the fluorescence for D, L types aminoacids was published by the Richard GI [8] and co-authors. However, authors in this particular publication used more complicated and difficult to perform method than the technique described by us.

Demonstrated above results might be useful tool for the quantative as well as qualitive analysis of glutamic acid as well as valine during pharmaceutical controlling experiments. These test system is easy to perform and cheap in comparison with the experiments performed by the utility of NMR as well as different types of HPLCs.

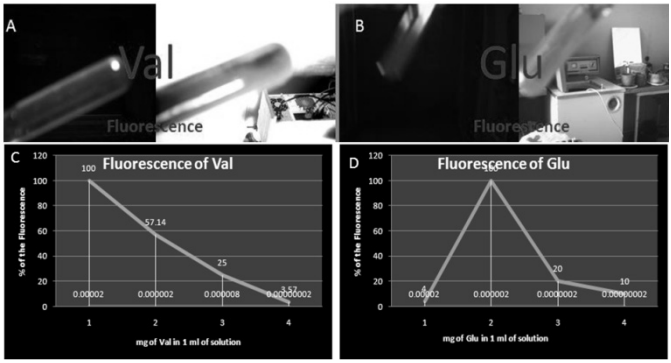


Fig.1. Visualization as well as measurement of the Glu and Val's fluorescence intensity in the water solution. Glutamic acid as well as valine (0.002 g) were mixed with the Resorcinol (0.002 g) and 5 drops of concentrated sulfuric acid. The mixture was heated until the visualization of the greenish brown coloring. Further, the samples were cooled by the addition of the 5 ml water and 5 ml of 28% of the ammonium solution. The mixture obtained redish-violet coloring with the green fluorescence (A and B). The stock solutions of amino acids were dissolved and the standard curves based on the measured intensity of fluorescence (Fluorometer, Perkin Elmer) were built (C and D).

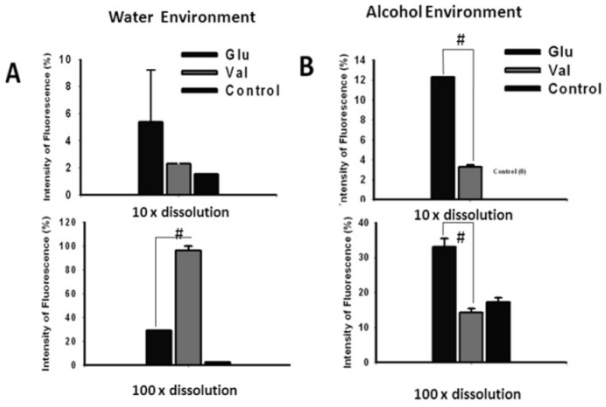


Fig.2. Estimation of fluorescence intensity for valine and glutamic acid in water and alcohol environments. Glutamic acid as well as valine (0.002 g) were mixed with the resorcinol (0.002 g) and 5 drops of concentrated sulfuric acid. The mixture was heated until the visualization of the greenish brown coloring. Further, the samples were cooled by the addition of the 5 ml water and 5 ml of 28% of the ammonium solution. The mixture obtained redish-violet coloring with the green fluorescence. Further, these stock solutions were dissolved for 10x and 100x in the water (A) and alcohol (B) and the intensity of fluorescence was measured (Fluorometer, Perkin Elmer). Statistical analysis were performed by the utility of t-student test as well as ONE-WAY-ANOVA. Results were statistically significant when the $p < 0.05$.

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ՎԱԼԻՆԻ ԵՎ ԳԼՅՈՒՏԱՄԱԹՎԻ ԴԵՂԱԳՈՐԾԱԿԱՆ ԱՆԱԼԻԶԻ ՄԵԹՈԴ՝ ՀԻՄՆՎԱԾ ՎԵՐՋԻՆՆԵՐԻՍ ՖԼՅՈՒՈՐԵՍՑԵՆՑԻԱ ԱՐՏԱԶԱՏԵԼՈՒ ՈՒՆԱԿՈՒԹՅԱՆ ՎՐԱ

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Կենսաբանական գիտությունների թեկնածու,
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Ա. Ա. ՍԻՄՈՆՅԱՆ
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L-Վալինը մի միացություն է, որը ներմուծվում է օրգանիզմ պարենտերալ եղանակով և արտադրվում է մի շարք երկրներում (Aminoven, Գերմանիա Highmix, Ռուսաստան և այլն): Այս ամինաթթուները կարող են որոշվել Հակադարձ փազային բարձր արդյունավետությամբ օժտված հեղուկային քրոմատոգրաֆիայի մեթոդով, նախապես քիմիական մոդիֆիկացիայի ենթարկելով ամինաթթուները և ստանալով իզոթիոցիանատի ածանցյալներ: Սակայն այս մեթոդը ժամանակատար է և դժվար իրականացվող: Մեկ այլ ամինաթթու՝ գլյուտամինաթթուն, մի դեղամիջոց է, որը բարելավվում է գլխուղեղի արյան շրջանառությունը և արտադրվում է մի շարք երկրներում, որոնց թվին են պատկանում Շվեդիան, Գերմանիան, Ռուսաստանը (Kabiven Central, Infesol 100, Eltacine): Գլյուտամաթթվի հիմնական տարբերակման ռեակցիան է հանդիսանում վերջինիս փոխազդեցությունը ռեզորցինոլի հետ, որի հետևանքով արտազատվում է ֆլյուորեսցենցիա: Համաձայն Ա.Պ. Արզամասցևի (Դեղագիտական քիմիայի մեթոդները) աշխատանքի՝ կանաչ ֆլուորեսցենցիան հատուկ է միայն գլյուտամինաթթվին, սակայն կիրառելով նույն մեթոդը վալինի տարբերակման համար՝ մենք հայտնաբերեցինք, որ վերջինս նույնպես օժտված է կանաչ ֆլուորեսցենցիայով, որը ավելի արտահայտված է ջրային միջավայրում և ոչ թե սպիրտային: Այս երկու միացությունների ֆլյուորեսցենցիայի որոշումը կարող է հանդիսանալ արագ մեթոդ վերջիններիս առկայությունը և քանակությունը որոշելու համար: Այս աշխատանքում մենք ներկայացնում ենք նոր, արագ մեթոդ վալինի առկայությունը և քանակությունը որոշելու համար:

Առանցքային բառեր՝ գլուտամինաթթու, նոր մեթոդ, ֆլյուորեսցենցիա, տարբերակում, ռեզորցինոլ

Որոշիչ բառեր՝ գլյուտամինաթթու, ռեզորցին, վալին, դեղ. քիմիա, որակական տարբերակում

МЕТОД ФАРМАЦЕВТИЧЕСКОГО АНАЛИЗА ДЛЯ ИДЕНТИФИКАЦИИ ВАЛИНА И ГЛЮТАМИНОВОЙ КИСЛОТЫ НА ОСНОВАНИИ ОПРЕДЕЛЕНИЯ ИХ ФЛЮОРЕСЦЕНТНОЙ СПОСОБНОСТИ

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L- Валин является соединением, которое вводится в организм парентеральным способом и продуцируется рядом стран (Aminoven, Германия; Highmix, Россия; etc.). Количественно это соединение может быть определено методом обратно-фазовой высокоэффективной жидкостной хроматографии после модификации аминокислот и получением изотиоцианатных производных этого соединения. Предколонковая модификация является трудоемкой и долгой методикой. Другая аминокислота-глутаминовая, используется в медицине для улучшения мозгового кровообрашения и производится рядом стран, в число которых входят Швеция, Германия, Россия (Kabiven Central, Infesol 100, Eltacine). Основной реакцией идентификации является взаимодействие этой аминокислоты с резорцинолом, в результате чего возникает зеленая флюоресценция. Согласно Арзамасцеву А.П. (Методы фармацевтической химии), реакция возникновения зеленой флюоресценции специфична только для глютаминовой кислоты. Однако мы выявили, что валин можно определить тем же методом. Более того, валин обладает более выраженной флюоресценцией, чем глютаминовая кислота в водной среде, но не в алкоголе. Этот метод может служить основой для идентификации и количественного определения как валина, так и глютаминовой кислоты быстрым, экспресс- методом. В этой работе мы предлагаем новый, быстрый метод качественного и количественного определения валина.

Ключевые слова: глютаминовая кислота, валин, качественная идентификация, резорцин, фарм химия