DEVELOPMENT AND VALIDATION OF LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY METHOD FOR DETERMINATION OF RIVAROXABAN IN PLASMA SAMPLES

Maja Miličević[1], Milkica Crevar[1*], Branka Ivković[1], Jelena Džudović[2]

[1]University of Belgrade – Faculty of Pharmacy, Department of pharmaceutical chemistry, Belgrade, Serbia
[2]Military Medical Academy, National Poison Control Center, Belgrade, Serbia

*milkica.crevar@pharmacy.bg.ac.rs

Rivaroxaban belongs to the group of direct oral anticoagulants (DOAC), drugs used to prevent and treat venous thrombosis and venous thromboembolism. Drugs from this group are considered safer than vitamin K antagonists. In case of overdose, their most significant side effect is bleeding. Given the great toxicological significance, it is very important to develop an analytical method for quantification of this drug in biological samples. The liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the determination of rivaroxaban content in plasma samples was optimized and validated. Plasma samples were prepared by protein precipitation with cold acetonitrile. Carbamazepine was used as an internal standard. The analysis was performed on an Infinity Lab Poroshell 120 EC-C18, 4.6 ×100 mm, 2.7 μm chromatographic column. The mobile phase consisted of acetonitrile and 0.1% formic acid (50:50, v/v), at a flow rate of 400 μL/min and a column temperature set at 30°C. The autosampler temperature was 4°C. The injection volume was 10 μL. Detection of analytes and internal standard was performed in multireaction monitoring mode (MRM), at the following ion transitions: 437>145 (m/z) for rivaroxaban, and 237>194 (m/z) for the internal standard. The optimized method was validated and the obtained parameters indicate that the method is sensitive, specific, selective, precise and accurate. The samples were stable under the tested conditions. A validated method has been used to determine the concentration of rivaroxaban in plasma samples of patients with atrial fibrillation who were hospitalized under strict medical supervision. Obtained concentrations were in the expected range.

RAZVOJ I VALIDACIJA METODE TEČNE HROMATOGRAFIJE SPREGNUTE SA MASENOM SPEKTROMETRIJOM ZA ODREĐIVANJE SADRŽAJA RIVAROKSABANA U UZORCIMA PLAZME

Maja Miličević[1], Milkica Crevar[1*], Branka Ivković[1], Jelena Džudović[2]

[1]Univerzitet u Beogradu – Farmaceutski fakultet, Katedra za farmaceutsku hemiju, Beograd, Srbija
[2]Vojnomedicinska akademija, Nacionalni centar za kontrolu trovanja, Beograd, Srbija

* milkica.crevar@pharmacy.bg.ac.rs

Rivaroksaban pripada grupi direktnih oralnih antikoagulanasa (DOAK), lekova koji se koriste za prevenciju i lečenje venske tromboze i venske tromboembolije. Lekovi iz ove grupe se smatraju bezbednijim od antagonista vitamina K. U slučaju predoziranja, njihov najznačajniji neželjeni efekat je krvarenje. S obzirom na veliki toksikološki značaj, veoma je važno postojanje analitičke metode za određivanje sadržaja ovog leka u uzorcima biološkog materijala. Optimizovana je i validirana metoda tečne hromatografije spregnute sa masenom spektrometrijom (LC-MS/MS) za određivanje sadržaja rivaroksabana u uzorcima plazme. Uzorci plazme su pripremani metodom precipitacije proteina koja je vršena hladnim acetonitrilom. Kao interni standard korišćen je karbamazepin. Analiza je izvršena na Infinity Lab Poroshell 120 EC-C18, 4,6×100 mm, 2,7 μm hromatografskoj koloni. Mobilna faza se sastojala od acetonitrila i 0,1% mravlje kiseline (50:50, v/v), pri protoku od 400 μL/min i temperaturi kolone podešenoj na 30°C. Temperatura autosemplera je bila 4°C. Injekciona zapremina je bila 10 μL. Detekcija analita i internog standarda je izvršena u multireakcionom monitoring modu (MRM), pri sledećim jonskim prelazima: 437>145 (m/z) za rivaroksaban, odnosno 237>194 (m/z) za interni standard. Optimizovana metoda je validirana i dobijeni parametri ukazuju da je metoda osetljiva, specifična, selektivna, precizna i tačna. Uzorci su stabilni pri ispitivanim uslovima. Validirana metoda je primenjena za određivanje koncentracije rivaroksabana u uzorcima plazme pacijenata sa atrijalnom fibrilacijom, koji su bili hospitalizovani, pod strogim medicinskim nadzorom. Određene koncentracije su bile u očekivanom opsegu.