

SAMPLE PREPARATION AND HPLC ANALYSIS OF METFORMIN IN PLASMA OF WOMEN WITH POLYCYSTIC OVARY SINDROME

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In recent years, polycystic ovary syndrome (PCOS) is a common endocrinopathy that affects approximately 5% to 10% of women at reproductive age (1). Metformin (MTF) or dimethyl biguanide is oral antidiabetic drug which is also being used in the treatment of PCOS, but its therapeutic regimens for this purpose are still not well standardized (2). Sample preparation is important step in bioanalysis and the extraction of MTF from biological samples is somewhat complicated by the polar nature of this molecule. The aims of this study were optimizing sample preparation procedure, as well as, evaluation and validation of HPLC method for determination of metformin in plasma obtained from women with PCOS. Blood plasma samples were collected from women with PCOS (n=54), 2-3h after the dosing. Extractions with different polar organic solvents and water solutions of acids (methanol, ethanol, acetonitrile, 10% HClO₄, 10% trichloroacetic acid) were performed, followed by centrifugation and filtration through 0.45 µm membrane filter. Twenty microliters of supernatant was injected on Zorbax-NH₂ column and the separation of MTF was achieved at 30 °C using isocratic elution. The mobile phase was consisted of 100% acetonitrile with flow rate 0,725 mL/min. The detection was monitored at 232 nm and quantification was done by calibration curve method. After optimization of experimental conditions, the highest values for recovery (over 92%) for MTF from blank plasma samples spiked with standard were obtained by preparation with small volume of acetonitrile. For optimized HPLC analysis, good linearity was found in the range of 0.1-5.0 µg/mL (r=0.9997), detection and quantification limits were 0.053 µg/mL and 0.175 µg/mL. Applying the chosen sample preparation procedure and HPLC analysis, the obtained concentrations of MTF in plasma of women with PCOS were in the range 1,24±0,07 µg/mL to 2,38±0,17 µg/mL, whereas precision was also satisfying (RSD<8.5%). The proposed method analysis was based on simple procedure by using one organic solvent and small volume of collected biological sample, as well as on the use of simple mobile phase and polar amino column. This combination of selected chromatographic conditions enabled very short retention time of MTF (around 3.7 min) and total time of one chromatographic run less than 6 min. Furthermore, satisfactory analytical parameters in terms of validation for the analysis of MTF in plasma of women with PCOS were obtained.

References

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PRIPREMA UZORAKA I HPLC ANALIZA METFORMINA U PLAZMI ŽENA SA SINDROMOM POLICISTIČNIH JAJNIKA

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Poslednjih godina sindrom policističnih jajnika (PCOS) je uobičajena endokrinopatija koja pogađa približno 5% do 10% žena u reproduktivnoj dobi (1). Metformin (MTF) ili dimetil-bigvanid je oralni antidijabetik koji se takođe koristi u lečenju PCOS-a, ali njegovi terapijski režimi u tu svrhu još uvek nisu dobro standardizovani (2). Priprema uzoraka je važan korak u bioanalizi, a ekstrakcija MTF iz bioloških uzoraka donekle može biti komplikovana zbog polarne prirode ovog molekula. Ciljevi ovog istraživanja bili su optimizacija postupka pripreme uzoraka, kao i evaluacija i validacija HPLC metode za određivanje metformina u plazmi uzorkovanoj od žena sa PCOS. Uzorci krvne plazme sakupljeni su od žena sa PCOS (n=54), 2-3 sata nakon doziranja. Ekstrakcije su izvršene različitim polarnim organskim rastvaračima i vodenim rastvorima kiselina (metanol, etanol, acetonitril, 10% HClO₄, 10% trihlorosirćetna kiselina), nakon čega je usledilo centrifugiranje i filtriranje kroz membranski filter od 0,45 mm. Dvadeset mikrolitara supernatanta je injektovano na Zorbax-NH₂ kolonu i razdvajanje MTF je postignuto na 30°C izokratnim režimom rada. Mobilna faza se sastojala od 100% acetonitrila sa protokom od 0,725 mL/min. Detekcija je praćena na 232 nm, a kvantifikacija je izvršena metodom kalibracione krive. Nakon optimizacije eksperimentalnih uslova, najveće vrednosti za MTF (preko 92% od očekivanih) iz blank uzoraka plazme kojima je dodat standard određenih koncentracija dobijene su korišćenjem male zapremine acetonitrila. Za optimizovanu HPLC analizu, dobra linearnost je nađena u opsegu koncentracija od 0,1-5,0 µg/mL (r=0,9997), dok su limit detekcije i kvantifikacije iznosili 0,053 µg/mL i 0,175 µg/mL. Primenom odabranog postupka pripreme uzoraka i HPLC analize, određene koncentracije MTF u plazmi žena sa PCOS bile su u rasponu od 1,24±0,07 µg/mL do 2,38±0,17 µg/mL, dok je i preciznost bila zadovoljavajuća (RSD<8,5%). Predložena metoda zasnivala se jednostavnim postupku korišćenjem jednog organskog rastvarača i male zapremine prikupljenog biološkog uzorka, kao i na upotrebi jednostavne mobilne faze i polarne amino kolone. Ova kombinacija odabranih hromatografskih uslova omogućila je veoma kratko retenciono vreme za MTF (oko 3,7 min) i ukupno vreme jedne hromatografske analize za manje od 6 min. Takođe, dobijeni su i zadovoljavajući analitički parametri u smislu validacije za analizu MTF u plazmi žena sa PCOS.

Literatura

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