DEVELOPMENT OF TARGETED LC-MS/MS METHOD FOR ANALYSIS OF DICLOFENAC AND ITS MAIN METABOLITES IN RAT LIVER PERFUSION SOLUTION OBTAINED BY NEW TYPE OF EX VIVO PERFUSION SYSTEM

Aya Dweny[1], Tímea Körmöczi[1], Orsolya Kovács[2], Reza Samavati[3], Róbert Gáspár[2], Róbert Berkecz[1]

[1]Department of Medical Chemistry, University of Szeged, H-6720 Szeged, Dóm tér 8, Hungary
[2]Department of Pharmacology and Pharmacotherapy University of Szeged, H-6720 Szeged, Dóm tér 12, Hungary
[3]Department of Pharmacodynamics and Biopharmacy, University of Szeged, H-6720 Szeged, Eötvös utca 6, Hungary
e-mail: berkecz.robert@med.u-szeged.hu

Abstract

Isolated perfused rat liver (IPRL) is used as ex vivo simulation for more accurate evaluation of pharmacokinetic parameters of target drugs, production of their metabolites and their excretion in the bile. Compared to in vivo systems, IPRL avoids the clearance of any other organ and permits the control over the factors that may affect the hepatic metabolism, for example, the flow rate of the perfusate, pH, pressure, and concentration of chemicals.
This simple experimental technique, unlike the cultured cells, keeps the intactness of the liver, in other words, cells other than hepatocytes, drug transporters' distribution, as well as enzymes, are still affecting the metabolism which gives a realistic picture [1-2].
The portal vein of the anesthetized rat was cannulated and the perfusion solution saturated with carbogen (95% O_2 and 5% CO_2) was pumped through it. The solution was directed through the transected abdominal vein to the waste. A cannula was inserted into the inferior vena cava through the right heart atrium through which the perfusate leaved the liver after the ligation of the abdominal vein. The isolated liver was then placed into the buffer vapour chamber of the recirculated perfusion system and the perfusate samples were collected during different time intervals.
The main goal of our work was the development of targeted reversed-phase LC-MS/MS analytical method for the analysis of diclofenac (DF) and its main metabolites namely diclofenac-O-acyl glucuronide (Glu-DF) and 4-hydroxydiclofenac (4´OH-DF) in the perfusion solution of rat liver. We are planning to use diclofenac as a reference compound in the future investigation of metabolism of designer drugs.
DF and its metabolites were extracted by the optimized liquid-liquid extraction procedure. LC separation was achieved by gradient elution of the mobile phase consisted of 0.1% formic acid in water and 0.1% of formic acid in acetonitrile on a Luna Phenyl-Hexyl column at 50˚ C with a run time of 10 minutes. The Agilent 1100 LC system was coupled to the triple quadrupole TSQ 7000 mass spectrometer. The appropriate transitions were monitored during the region of elution of each analyte. The linearity range of DF was between 100 ng/mL and 40 μg/mL. The related matrix effect, recovery and process efficiency were successfully evaluated.

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References

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