K18 Ultra-Fast Determination of Metformin in Plasma by Hydrophilic Interaction Chromatography: Application in a Fatal Case of Metformin Self-Poisoning

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The learning objective of this paper is to present an ultra-fast and accurate method for the determination of metformin in plasma by hydrophilic interaction chromatography on a special stationary phase. This paper will present the toxicological results, together with the clinical data in a fatal case of lactic acidosis due to a self-poisoning by metformin. The authors will demonstrate that assay of this biguanide in plasma may contribute towards the differential diagnosis of the acidosis.

Metformin (1,1-dimethylbiguanide, hydrochloride or p-chlorophenoxyacetate salt) is an oral hypoglycemic agent, widely used in France and Europe in the treatment of type II non-insulin dependent diabetes mellitus. This medication is considered safe if not used in the presence of contraindications. Daily oral doses range from 200-1500 mg as metformine base and even at doses higher than 85 g at once, no hypoglycemia was observed. The main adverse effect at high dose is an extremely severe lactic acidosis. Describe here a fatal case of metformin self-poisoning with a severe lactic acidosis and a special ultra-fast analytical method by HydrophILic Interaction Chromatography (HILIC) with photodiode array detection.

Case report: A 42-year-old Caucasian male was admitted to hospital for confusion and acute abdominal pain. He has a ten-years history of non-insulin dependent diabetes mellitus and received metformin therapy. However because of inadequate glycemic control under biguanide in this mentally deficient patient, metformin was stopped one year ago and insulin therapy was used, together with ibuprofen and tiaprofenic acid. At the admission, the patient was conscious but confuse and he argued attempting suicide with an unknown amount of metformin. He suffered of oliguria, important dehydratation, hypothermia, hypotension, renal failure and lactic acidosis with normal glucose (pH 6.88, bicarbonate 2.9 mmol/L, lactate 27 mmol/L, creatinine 163 Imol/L, glucose 16.4 mmol/L). The treatment consisted in a mechanical respiratory support, correction of fluid deficits, treatment of hypothermia and correction of acidosis by hyperventilation. Despite these intensive care, the patient developed an acute respiratory distress syndrome, anuria and shock. He died 34 hours after admission. A blood sample was obtained just before death.

Analytical conditions : Metformin is a very little polar molecule and so is very difficult to extract by classical organic solvents and to assay by reverse phase liquid chromatography (HPLC) because of a short retention time on octyl or octadecyl hydrophobic phases. HILIC was chosen to perform a fast and accurate method to determine metformin in plasma, because this method is well suited for the separation of little polar molecules. The HILIC column (200 x 4.6 mm) contains a poly(2-hydroxyethylaspartamide)-silica stationary phase (PolyHydoxy Ethyl A, PolyLC, USA)with 5Im particules. Mobile phase was acetonitrile : phosphoric acid (65 ; 35, v/v) at pH 2.8, with a flow-rate at 1.5 mL/min. Metformin was detected by a diode array detector at 234.6 nm (Waters 996). Extraction of metformin from plasma was very easy and fast, just adding 15 IL diluted perchloric acid to 250 IL plasma in order to precipitate the proteins.

Results and discussion: The mean retention time of metformin is 2.90 +/- 0.24 min. The linearity of the method is very good from 0.1 to 400 Ig/mL (r^2 = 0.99). The limit of detection is 0.02 Ig/mL, the limit of quantification is 0.1 Ig/mL. Recovery from plasma is excellent, reaching 99.5%. The authors verified that there is no analytical interference between metformin and other oral hypoglycemic medications (glibenclamide, glicazide, glipizide and benfluorex) : they are all eluted in the solvent front between 1.1 and 1.3 min.. Intra-day and inter-day variabilities are higher at low concentrations (cv = 20% at 0.25 and 1 Ig/mL) than at higher concentrations (cv = 2% at 50, 200 and 400 Ig/mL). Plasma metformine concentration was 188 Ig/mL. International literature syas that therapeutic blood concentrations range from 0.75-3 Ig/mL and toxic concentrations from 5-250 Ig/mL. However, the prognosis of metformin poisoning mainly depends on the concurrent pathology and administration of other medications: that is what was observed in this patient (dehydratation and two non-steroidal antiinflammatory drugs

Conclusion: The best HPLC analytical solution for the determination of polar xenobiotics is to use polar stationary phase eluted with an aqueous-organic mobile phase (HILIC). This technique appears to be well suited to determine metformin in plasma and it was applied in a fatal case of metformin intoxication. The developed method is ultrafast (less than 20 min) and accurate. As far as the symptoms of biguanide poisoning are non-specific, diagnosis of metformin intoxication may be improved by a rapid determination of the drug in blood.

Metformin, Hydrophilic Interaction Chromatography, Poisoning

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