Online Data Suplement

Prostaglandin E_2 systemic production in asthma patients with and without aspirin hypersensitivity.

Lucyna Mastalerz, MD, PhD, Marek Sanak, MD, PhD, Agnieszka Gawlewicz-Mroczka, MD, Anna Gielicz, MSc, Adam Ćmiel*, PhD, Andrew Szczeklik MD, PhD

Methods

Briefly, aliquot of urine (0.5 mL) was diluted with distilled water up to 2 mL. Fifteen ng of tetranor-PGEM-d_6 was added as an internal standard. The solution was acidified with 1N hydrochloric acid to pH 3.0 and prostaglandins were extracted twice with (1 and 1.5 mL) ethyl acetate. The organic phases were combined, dried over sodium sulfate and evaporated to dryness under stream of nitrogen.

The residue was derivatized at 40^{o}C to form pentafluorobenzyl ester (PFB-ester) with 40μL of 25% pentafluorobenzyl bromide (PFBBr) and 40μL of 2.0% N,N-diisopropylethylamine (DIPE) in acetonitrile. Acetonitrile was subsequently evaporated and the residue solved in 30 μL of methanol and subjected to further purification by thin layer chromatography (TLC) on LK6D silica gel 60Å in 13 cm distances. Solvent system for chromatography was ethyl acetate/n-heptan=80/20 v/v. Substances of interest were scraped off between 6 and 7 cm and eluted from silica gel with 1 mL of ethyl acetate. Organic solvent was evaporated and residue derivatized with methoxyamine hydrochloride to form O-methyloxime (MOX). Then trimethylsilyl-ether (TMS-ether) was formed with 15 μL of bis(trimethylsilyl)trifluoroacetamide (BSTFA) in a presence of pyridine (15 μL) for 5 min at 40^{o}C.

Derivatization agents were evaporated. The residue (PFB-MOX-TMS-derivatives of tetranorPGE-M) was extracted with 800μL n-heptane. After evaporation n-heptane the sample was dissolved in 10μL of undecane. 1-2 μL of the solution was injected on GC-MS. The ions of 637 and 643 mass-to-charge for tetranor-PGE-M and tetranor-PGEM-d_6 respectively were recorded in selected ion monitoring of negative ion chemical ionisation mode.

Amounts of tetranor-PGE-M were expressed in nanograms per mg of creatinine, after recalculation for a deuterated compound to compensate the loss during preparation.