Fast HPLC-DAD method for simultaneous phenotyping of CYP2C19 and CYP3A4 through the determination of omeprazole and two of its metabolites in human plasma samples

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AIMS: The metabolic profile of a certain individual can be evaluated by genotyping or phenotyping of certain enzymes. Besides the applicability of genotyping, phenotyping procedures can be valuable to know the activity of a metabolizing enzyme at a certain moment, taking in consideration that sometimes genotype and phenotype are not concordant. One usual method to access the individual metabolic capability of a certain individual is through the administration of a single dose of a probe drug, mainly metabolized by the CYP enzyme to be evaluated. Omeprazole (OME) has shown to be a useful probe for CYP2C19, being converted to 5-hydroxy-omeprazole (HOME), and CYP3A4, being converted to omeprazole sulphone (OMES). The metabolic ratios for both pathways are used to classify individuals as poor (PM), extensive (EM) or fast metabolizers (UM). We developed a fast HPLC-DAD for the simultaneous determination of HOME, OMES and OME in human plasma samples.

METHODS: Samples of 1 mL were alkalinized with 0,5 mL tris buffer pH 9,5 and extracted with 6 mL of ethyl acetate. The organic phase was dried and recovered in mobile phase, which consisted of a mixture of acetonitrile and phosphate buffer pH 7.6 (24:76). Twenty microlitrers were injected in a Shimpack C18 column (15 × 0,46 mm). Flow rate was 1.2 mL/minute. Retention times were 4,0 minutes for HOME, 11.2 minutes for OME and 13.0 minutes for OMES. Sulpiride was used as internal standard. The use of a DAD detector allowed spectral confirmation and purity evaluation of all detected peaks. The volunteers of the phenotyping study, after a overnight fasting, took an oral dose of 20 mg of omeprazole. Blood samples were drawn three hours after ingestion. Preliminary results of the phenotyping of 31 individuals showed the presence of 1 UM for CYP2C19, 1 UM for CYP3A4, 3 PM for CYP2C19 and 2 PM for CYP3A4. All other volunteers were considered as EM.

RESULTS: The calibration curve was linear in the range of 25-1000 ng/mL. Accuracy was in the range of 94-104%, LOD was 7 ng/mL, LOQ was 21 ng/mL, precision was in the range of 2,3 – 8,6 %, specificity and stability were acceptable.

CT-p-11

CONCLUSIONS: Evaluation of the activity of CYP2C19 and CYP3A4 using omeprazole as a probe drug can be a valuable tool to optimize the dosage regimen for drugs metabolized by these two enzymes. Previous work from Kirchheiner (2001) had proposed the individualization of dosage regimen of psychotropic drug based on CYP2D6 and CYP2C19 genotypes. In this approach, PM should receive lower doses and UM must require higher doses of drug metabolized by the specific polymorphic enzymes. The same approach can be used with phenotyping data, specially when using metabolic rations obtained on routine therapeutic drug monitoring. Further studies will be performed by our group on the correlation of CYP2C19 phenotype determined with omeprazole with amitriptyline metabolic ratios and effectiveness on fibromialgy therapy. The proposed method is fast, reliable and sensitive enough to classify the metabolic status of poor, extensive and fast metabolizers. The method is currently being used in the phenotyping of a group of 120 healthy Brazilian volunteers.

KEYWORDS: *CYP2C19, CYP3A4, phenotyping, omeprazole, 5-hydroxyomeprazole, omeprazole sulphone, HPLC-DAD*

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