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Analysis of omeprazole, midazolam and hydroxy-metabolites in plasma using liquid chromatography coupled to tandem mass spectrometry

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Abstract

A method has been developed and validated for the quantitation of midazolam, alphahydroxy-midazolam, omeprazole, and hydroxyomeprazole from one 250 μL sample of human plasma using high performance liquid chromatography coupled to tandem mass spectrometry. The method was validated for a daily working range of 0.400–100 ng/mL, with limits of detection between 2 and 15 pg/mL. The inter-assay variation was less than 15% for all analytes at four control concentrations and the samples were stable for three freeze–thaw cycles under the analysis conditions and 24 h in the post-preparative analysis matrix. This method was used to analyze samples in support of clinical studies probing the activity of the cytochrome P-450 enzyme system.

Keywords: Omeprazole; Midazolam; Analysis; LCMS; CYP2C19

1. Introduction

The use of probe substrates for evaluating the effects of various factors such as genetics, environment, gender, and xenobiotics on in vivo Cytochrome P-450 (CYP) enzyme activity is an increasingly common practice [1–4], and is an accepted indirect method for evaluating the metabolism and drug interaction potential of new compounds during drug development [4,5]. An ideal probe substrate should be selective for the enzyme being studied, be sensitive to changes in enzyme content or activity, require minimally invasive sampling, be nontoxic, and not directly affect the activity of the enzyme.

The most commonly recommended biomarker for indirectly measuring hepatic CYP3A4 activity is the total body clearance of intravenous midazolam (MDZ) [1,3,4,6]. Following intravenous administration, MDZ is selectively metabolized by the CYP3A subfamily, with CYP3A4 being the predominant catalyst. Thummel et al. [7] demonstrated an excellent correlation ($r = 0.93$, $p < 0.001$) between in vivo total midazolam clearance and hepatic CYP3A content measured ex vivo in liver transplant patients. Midazolam has met most of the putative criteria as a selective and sensitive probe for CYP3A activity [1,6–8], has demonstrated a small degree of intraindividual variability over 3 months, and is not affected by gender or menstrual cycle phase [9]. Midazolam has the advantages of intravenous administration to avoid pre-systemic metabolism, and low cost. In addition, changes in midazolam clearance may be clinically relevant since the drug is a commonly used sedative. The major disadvantages of MDZ as an in vivo CYP3A probe are the sedative effects, and the need to obtain multiple blood samples over 6–8 h. In addition, MDZ may not accurately reflect CYP3A4 activity in patients with a high hepatic extraction ratio, or altered protein binding of the drug [10].

Omeprazole (OPZ) has emerged as the preferred in vivo probe for determining CYP2C19 phenotype [1,4]. Oral

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mephenytoin, the preferred probe substrate in the past, is no longer available and has a risk of sedative adverse effects, especially in poor metabolizers [11]. The ratio of serum OPZ to 5′-hydroxyomeprazole (OH-OPZ) concentrations, also called the hydroxylation index (HI) has excellent concordance with CYP2C19 genotype [11–13], and is not influenced by gender or menstrual cycle phase [14]. In addition, the omeprazole HI is normally distributed among extensive metabolizers, potentially allowing more detailed studies within this group. The OPZ HI has been calculated using area under the curve (AUC) ratios for the parent and metabolite [11], or a single-point measurement at 2–3 h after the dose [11–13]. Omeprazole has the advantages of easy administration, the potential for single point measurement of the HI, and an excellent safety profile. Disadvantages of OPZ for CYP2C19 phenotyping include a high degree of intra-subject variability reported with the 2 h index [14], and a portion of subjects having undetectable parent or metabolite serum concentrations at the single point sampling time [3,14]. Use of a larger oral dose or use of AUC ratios may overcome the limitation of undetectable serum concentrations with single point methods, as well as better analytical methods.

The administration of multiple probe "cocktails" to determine the phenotype for several CYP enzymes in a single study visit is a common practice [2–4]. The development of assay methods for the simultaneous quantification of probe drugs and their major metabolites in plasma has the potential to simplify the performance of multiprobe studies by reducing sample volume requirements, assay time and costs. In this report we describe the development and validation of a sensitive and specific chromatographic method for the simultaneous quantification of MDZ, OPZ and their hydroxyl-metabolites. Structures of the analytes are shown in Fig. 1 [15].

The use of liquid chromatography coupled to tandem mass spectrometry (LC/MS/MS) has emerged as the developmental method of choice in supporting clinical and pre-clinical pharmacokinetic studies [16]. This is based on the ability of this technique to provide superior specificity, speed and detectability in complex matrices, as compared to commonly used high pressure liquid chromatography with ultra-violet absorbance detection (HPLC-UV) methods [16]. When comparing these two techniques, LC/MS/MS is able to analyze more compounds in less time, with a lower limit of quantitation.

However, with electrospray ionization (ESI) coupled to LC/MS/MS, the issue of unstable instrument response due to ion-suppression must be considered. For reliable quantitation, the belief that very little, if any, sample preparation is needed is typically untrue [17]. Therefore, it is critical that any method developed by LC/MS/MS for quantitation of clinical samples be thoroughly characterized, especially for accuracy in various matrices. This is increasingly important as a greater number of analytes of varying chemical properties are included in one analysis.

For this particular analysis, a review of the literature suggested that solution stability of the compounds should to be given particular consideration. It has been documented that OPZ, sparingly soluble in aqueous media, is unstable unless stored and handled at basic pH [18,19]. In studies investigating the mode of action of OPZ, it was presented that the molecule undergoes acid catalysis, and that the molecule has a half-life of only 1.4 h at pH 5.1, increasing to 38.5 h at pH 7.4 [20,21]. Another study noted that OPZ is stable at −20 °C for 1 month in plasma if the plasma is buffered at pH 8 [22]. This indicated a preferable use of basic media for OPZ analysis.

Second, a review of the literature revealed that MDZ can photo-degrade in aqueous solution, noting a 10% degradation in 1 h at pH 6.4. This decomposition was reduced by half as the media increased in acidity to pH 1.3 [23]. Also, solution pH dictates MDZ residence in open ring (acidic) or closed-ring form (neutral and basic pH). It has been observed that the open-ring form degrades more slowly when exposed to light than the closed ring form [23]. The effect of form on MDZ fragmentation was unknown at the outset of this tandem mass spectrometry development. This indicated a handling of MDZ in amber coated containers, with minimal exposure to light. Thus, consideration of the documented drug stability characteristics dictated the storage and solution conditions within this development.

2. Experimental

2.1. Chemicals

Omeprazole and formic acid were obtained from Sigma–Aldrich (St. Louis, MO, USA) Midazolam, alphahydroxymidazolam (OH-MDZ), and flurazepam were obtained from Lipomed (Cambridge, MA, USA). Hydroxy-omeprazole was donated from Astra–Hassle (Basel, Switzerland). Water, ammonium acetate, methanol, hexanes, and ethyl acetate were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Acetonitrile and ammonium hydroxide was obtained from VWR (South Plainfield, NJ, USA). All solvents used in sample preparation and chromatographic separations were of HPLC grade. Plasma for preparation of standards, quality controls, and blanks was obtained from The Interstate Blood Bank (Memphis, Tenn., USA).

2.2. Instrumentation

The LC/MS/MS system consisted of an Agilent 1100 series autosampler (Foster City, CA, USA) an Agilent 1100 series pump, an Agilent 1100 series degasser, and an Applied Biosystems PE/Sciex, API 3000 mass spectrometer (Foster City, CA, USA) equipped with a Turbo-ionspray source. The system was controlled through Analyst Software, version 1.1 from Applied Biosystems.