Optimisation of Chiral Separation of Omeprazole and One of Its Metabolites on Immobilized \(\alpha_1\)-Acid Glycoprotein Using Chemometrics

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Key Words
- Column liquid chromatography
- Experimental design
- Direct chiral resolution
- Omeprazole
- Hydroxy-omeprazole

Summary
A strategy for the optimisation of direct chiral separation of omeprazole and a metabolite, hydroxy-omeprazole, is described. A factorial design was used, where mobile phase pH, concentration of a mobile phase modifier, ionic strength and column temperature were tested as the variables and enantioselective retention, column efficiency and asymmetry factor as the responses. The experimental results were evaluated with multivariate analyses, which demonstrated that the column temperature and content of mobile phase acetonitrile were by far the most important variables. The enantiomers of omeprazole and one of its metabolites were baseline resolved within 15 minutes. The optimised chromatographic system was used for a separation of the enantiomers of omeprazole and its main metabolite in a patient plasma sample.

Introduction
Today most of the pharmacologically active substances on the market containing an asymmetric centre or plane are administrated to patients as racemates. Since the enantiomers may have different physiological effects in biological systems, it is of significance that both enantiomers could be determined and analysed, not only the racemate. Column liquid chromatography is a suitable technique used for this purpose [1, 2].

In this study a protein, \(\alpha_1\)-acid glycoprotein, immobilised to silica particles (Chiral-AGP®) was used as the chiral stationary phase [3]. This kind of chiral stationary phase can retain enantiomers by different adsorption mechanisms. Achiral sites contribute to the overall retention of the solutes, whereas the chiral sites contribute to the selective retention of the enantiomers. The relative amount of chiral and achiral sites provide the resulting enantioselectivity. Possible interactions between the enantiomers and the immobilised protein can be of electrostatic nature, hydrophobic interaction and hydrogen bonding [4]. In addition, sterical factors may also be of importance for the enantioselective recognition [3, 5, 6]. The Chiral-AGP® column has been used for enantioselective separations of several different classes of compounds, e.g. amines, carboxylic acids and aprotic solutes [5]. Enantioselective retention on protein-silica stationary phases has previously been regulated by the mobile phase pH, ionic strength, column temperature [4, 7-11], as well as charged and uncharged organic additives [12].

Earlier studies showed that ionic strength influenced the enantiomeric separations for hydrophilic compounds [4]. In order to obtain reasonable retention times as well as improve the enantioselectivity organic modifiers can be added to the mobile phase [5]. A previous study revealed that acetonitrile was a proper choice to separate the enantiomers of omeprazole on Chiral-AGP® within an acceptable retention time [11].

Omeprazole is a potent inhibitor of gastric acid secretion and is successfully used against acid-related diseases. Both enantiomers of omeprazole have this inhibitory effect, performed by a different mechanism than e.g. \(H_2\)-receptor blockers [13]. Omeprazole is transformed to intermediary products that selectively inhibit \(H^+, K^+-\)ATPase. This enzyme is responsible for the
gastric acid production and is located in the secretory membranes of the parietal cells in the stomach [14–16]. The number of metabolites are at least six, among which hydroxylated omeprazole is the main metabolite [17, 18].

The separation of the two chiral benzimidazoles, omeprazole (solute 1) and one of its metabolites, solute 2, structures in Figure 1, was optimized with experimental design and evaluated with multivariate analyses.

The mobile phase pH (5.7–7.2), concentration of acetonitrile (10–15 per cent v/v), column temperature (20–40 °C) and ionic strength (0.01–0.05) were the descriptor variables generating the responses, i.e. log-aritmed capacity factors ($k'$), separation factors ($c_0$), asymmetry factors ($Asf$) and column efficiencies ($N$).

**Experimental**

**Chemicals**

Acetonitrile, (gradient quality), methanol, (HPLC quality), sodium di-hydrogen phosphate (GR) and di-sodium hydrogen phosphate (GR) were obtained from Merck (Darmstadt, Germany). Omeprazole (solute 1), 5-methoxy-2-[[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole and solute 2, 5-methoxy-2-[[[(4-methoxy-3-methyl-5-hydroxymethyl-2-pyridinyl)methinyl]sulfinyl]-1H-benzimidazole, Figure 1, were synthesised by the department of Medicinal Chemistry at Astra Hässle AB (Mölndal, Sweden). Purified water was supplied by Elgastat Maxima from Elga (Wycomb, UK).

**Chromatography**

The chromatographic system consisted of a Binary LC Pump 250 (Perkin Elmer, Norwalk, USA), an AS3000 autosampler (Spectra Physics, San José, USA) and an UV-VIS-detector, (Chrompack, Middelburg, The Netherlands). The Chiral-AGP® column (100 x 4.0 mm, 5 µm), consisting of α1-acid glycoprotein immobilised to silica, was bought from ChromTech (Stockholm, Sweden). The temperature of the column and solvent reservoir was controlled by a water bath (LTD6, Grant, Cambridge, UK). The solutes were detected at 302 nm. The flow rate was 1.0 mL min⁻¹ unless stated otherwise. The injection volume was 20 or 50 µL and the sample concentration 0.3–50 µM. The pH in the phosphate buffer solution was measured with a PHM85 pH meter (Radiometer, Copenhagen, Denmark). The centrifugation of the plasma samples was performed by a Rotixa/AP (Heltich, Germany) at 2500 rpm. All the mobile phase compositions are given in Table I.

**Chromatographic responses**

The capacity factor ($k'$) was calculated using the equation,

$$k' = \frac{t_r}{t_0} - 1$$

(1)

where $t_r$ is the retention time for the compound and $t_0$ is the retention time for an unretained compound, in this study NO₃⁻. The separation factor ($\alpha$) was calculated using the equation,

$$\alpha = \frac{k'_2}{k'_1}$$

(2)

where $k'_2$ is the capacity factor for the last eluted enantiomer (the (S)-form) and $k'_1$ is the capacity factor for the first eluted enantiomer, (the (R)-form). The absolute configuration of (R)- and (S)-omeprazole has been determined by using X-ray crystallography [19]. Further, $\alpha_3 = \frac{a_3}{a_1}$ = the capacity factor for (R)-omeprazole divided with the capacity factor for the first eluted enantiomer of solute 2 (the (R)-form) and $\alpha_4 = \frac{a_4}{a_2}$ = the capacity factor for the last eluted enantiomer of solute 2 (the (S)-form), divided with the capacity factor for (R)-omeprazole.

The asymmetry factor was calculated using the equation,

$$Asf = \frac{b}{a}$$

(3)

where $b$ is the later half and $a$ is the former half of the bandwidth, measured at the baseline. The column efficiency, expressed as the number of theoretical plates was calculated using the equation,

$$N = \frac{16t^2}{w^2}$$

(4)

where $w$ is the bandwidth measured at the baseline [20].

**Statistical Methods**

A fractional factorial design (CCF, central composite facial design) with centerpoints was used to examine the