An LC-MS/MS Method for the Determination of 13 Antidepressants and Metabolites with Preliminary Data Comparing Plasma and Oral Fluid Concentrations

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AIMS: The aim of this study was to develop an LC-MS/MS method for the simultaneous analysis of amitriptyline, imipramine, clomipramine, fluoxetine, paroxetine, fluvoxamine, sertraline, citalopram and venlafaxine and some of their metabolites (nortriptyline, desipramine, norclomipramine and norfluoxetine). This methodology is being used to assess the relationship of the antidepressant concentrations found in plasma and oral fluid.

METHODS: The sample (200 µL of plasma or oral fluid conditioned with sodium acetate buffer pH 3.6) was extracted with an automated solid-phase extraction system, using mixed mode OASIS MCX cartridges. Chromatographic separation was performed using a reverse phase Sunfire C18 IS column (20 x 2.1 mm, 3.5 µm). The mobile phase consisted of acetonitrile and 2 mM ammonium formate used in a gradient mode. Under these conditions, all of the compounds eluted in less than 5 minutes with a total run time of 8 minutes.

To assess the degree of correlation of antidepressant concentrations between both types of specimens, plasma and oral fluid samples were collected from patients on antidepressant treatment in two different weeks. Oral fluid samples were collected by direct spitting.

RESULTS: The method was fully validated, including linearity (2-4 to 500-1000 ng/mL), within-day and between-day precision (CV < 15%), accuracy (MRE < 15%), limit of detection (0.5 ng/mL), limit of quantitation (2 - 10 ng/mL), recovery, relative ions intensity, matrix effect and stability after 3 freeze/thaw cycles (CV < 20%, except for sertraline (CV = -33.4% in oral fluid at the highest studied concentration)). The patients whose samples were processed were in treatment with venlafaxine (n = 6), citalopram (n = 6), paroxetine (n = 4), sertraline (n = 3), fluoxetine (n = 3), amitriptyline (n = 2) and clomipramine (n = 1). In this preliminary study, samples collected on only two different occasions were analyzed to evaluate which compounds may provide a good correlation. Correlation between plasma and oral fluid concentrations was calculated by linear regression for each compound individually. Data for the different compounds indicate that there is not a good correlation for any of the them when the results were analyzed interindividually. The best result was for fluoxetine ($r^2 = 0.739$) and the worst for citalopram ($r^2 = 0.0114$). Neither was any correlation found for any of the compounds when data were analyzed intraindividually, except for venlafaxine ($r^2 > 0.90$) in five out of the six patients).

CONCLUSIONS: A fast method was developed and fully validated for the analysis of the most commonly marketed antidepressants in plasma and oral fluid. To our knowledge, this is the first LC-MS/MS method that allows the analysis of these compounds in oral fluid samples. A research project to evaluate the relationship of antidepressant concentrations between these two matrices has been started. Preliminary data when analyzing plasma and oral fluid samples intraindividually in two consecutive weeks indicate a possible good correlation between venlafaxine levels in both matrices. Our next step will be to extend the number of samples from patients on venlafaxine treatment to confirm or rule out a good correlation between plasma and oral fluid.

Keywords: Antidepressants, Plasma, Oral fluid