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**AIM:** The aim of this study was to develop a simple, rugged and high-throughput method for rapid and simultaneous bio-analysis of the main antidepressants prescribed in Belgium using 50µL of plasma.

**METHODS:** An on-line solid phase extraction (SPE) method coupled to LC-MS/MS was developed for the simultaneous analysis of amitriptyline, imipramine, clomipramine, trazodone, fluoxetine, paroxetine, fluvoxamine, sertraline, citalopram and venlafaxine and some of their metabolites (nortriptyline, desipramine, norclomipramine and norfluoxetine). Ionization was achieved using electrospray in the positive ionization mode (ESI+). Fifty µL of sample previously diluted with 950µL of 0.1% formic acid were extracted using Oasis MCX Prospekt-2 disposable cartridges (Waters, Milford, MA, USA). Chromatographic separation of all the compounds was achieved within 15 minutes with a Phenomenex Gemini C18 (150 × 2 mm, 5 µm) analytical column, using a gradient consisting of ammonium bicarbonate buffer 10 mM, pH 10 and acetonitrile. The total run time was 20 min, including solid phase extraction and chromatographic separation.

**RESULTS:** the method was fully validated, including linearity (10-1000ng/mL), within-day and between-day precision (CV<15%) and accuracy (MRE<15%) for QC samples at 40, 200 and 800ng/mL, limit of quantification (10ng/mL), matrix effect and stability in the autosampler (24 and 48 hours) and after 3 freeze/thaw cycles. Selectivity of the method against endogenous interferences was verified by analysing blank plasma samples from 6 different sources. The method was applied to the analysis of 11 real plasma samples, previously screened using a routine HPLC-DAD method for systematic toxicological analysis.

**CONCLUSIONS:** To our knowledge, so far this is the first on-line SPE method with single use cartridges, coupled to LC-MS/MS which allows the detection of the main antidepressants in one single injection. The analytical results demonstrate the feasibility of this technique for high throughput and fully automated analysis, without compromising specificity and sensitivity.

**KEYWORDS:** *Antidepressant, On-line SPE, LC-MS/MS*

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