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Comparison of electron and chemical ionization modes by validation of a quantitative gas chromatographic–mass spectrometric assay of new generation antidepressants and their active metabolites in plasma

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Abstract

A gas chromatographic–mass spectrometric method (GC–MS) for the simultaneous determination of the 'new' antidepressants (mirtazapine, viloxazine, venlafaxine, trazodone, citalopram, mianserin, reboxetine, fluoxetine, fluvoxamine, sertraline, maprotiline, melitracen, paroxetine) and their active metabolites (desmethylmirtazapine, *O*-desmethylvenlafaxine, *m*-chlorophenylpiperazine, desmethylcitalopram, didesmethylcitalopram, desmethylmianserin, desmethylfluoxetine, desmethylsertraline, desmethylmaprotiline) in plasma using different ionization modes was developed and validated. Sample preparation consisted of a strong cation exchange mechanism and derivatisation with heptafluorobutyrylimidazole. The GC separation was performed in 24.8 min. Identification and quantification were based on selected ion monitoring in electron (EI) and chemical ionization (CI) modes. Calibration by linear and quadratic regression for electron and chemical ionization, respectively, utilized deuterated internal standards and a weighing factor $1/x^2$. Limits of quantitation were established between 5 and 12.5 ng/ml in EI and positive ionization CI (PICI), and 1 and 6.25 ng/ml in negative ionization CI (NICI). During validation stability, sensitivity, precision, accuracy, recovery, and selectivity were evaluated for each ionization mode and were demonstrated to be acceptable for most compounds. While it is clear that not all compounds can be quantitated either due to chromatographic (trazodone) or derivatisation problems (*O*-desmethylvenlafaxine), this method can quantitate most new antidepressants (ADs) in the therapeutic range using EI. PICI and NICI lead to higher selectivity. Moreover, NICI is of interest for small sample volumes and high sensitivity requirements. This paper draws the attention to the pros and cons of the different ionization modes in the GC–MS analysis of these antidepressants in plasma.

Keywords: Chemical ionization; Depression; Metabolites; Gas chromatography; Validation; Plasma

1. Introduction

Depression is a chronic or recurrent mood disorder that affects economic and social functions of about 121 million people worldwide, and can eventually lead to suicidal behaviour [1,2]. Before 1980, depression was treated using tricyclic antidepressants, monoamine oxidase inhibitors and lithium. However, according to the Cognos Plus Study 11 [3], the 'new' generation antidepressants (ADs) are the most prescribed antidepressant drugs in the seven major markets (Japan, USA, France, United Kingdom, Italy, Spain, Germany) nowadays. The 'new' generations include the Selective Serotonin Reuptake Inhibitors (SSRI's: fluoxetine, fluvoxamine, sertraline, paroxetine and citalopram), the Selective Noradrenalin Reuptake Inhibitors (reboxetine and viloxazine), the Serotonin and Noradrenalin Reuptake Inhibitors (venlafaxine), the Noradrenergic and Specific Serotonergic antidepressants (mirtazapine and mianserin), and the Serotonin-2 antagonists and Reuptake inhibitors such as trazodone [4–9].

New antidepressant drugs have a wide therapeutic range but an unclear plasma concentration–effect relationship, and, because therapeutic ranges seem quite broad, a notion of low toxicity is generally accepted. However, monitoring of these antidepressants can provide cost-effective, rational use of

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psychiatric drugs for special patient groups such as elderly, patients with liver and kidney impairment, patients with poor metabolism by the cytochrome P 450 (CYP 450) enzyme system and with co-medication with inhibitors and inducers of those enzymes [10–15]. In addition, monitoring of patient compliance is of interest. Both the parent compound and the active metabolites need to be determined as the latter also contribute to the overall therapeutic and toxic effect. In addition, metabolites can give extra information about the time of ingestion, the metabolic capacity, and compliance.

Over the years, several chromatographic methods have been developed for the determination of these ADs in biological matrices. These methods include capillary electrophoresis [16,17], high performance liquid chromatography with ultraviolet (UV) [18–21], fluorescence [22,23] or mass spectrometric detection [24–26], as well as gas chromatography combined with nitrogen–phosphorus [27,28] or mass detection (GC–MS) [29–32]. In clinical toxicology, GC–MS is still the method of choice as it is sensitive and selective, providing the best separation power for compounds that are volatile under GC conditions. Previously published multi-analyte GC–MS procedures are used for screening or quantification of several SSRI's. Our proposed method can be used for the simultaneous quantification of most new generation antidepressants and their metabolites. Moreover, these published methods use the electron ionization (EI) mode and do not compare with chemical ionization modes (CI). EI is the traditional method for comprehensive screening procedures, allowing identification of unknown compounds by comparison of their mass spectrum with a large collection of reference mass spectra in commercially available libraries. In addition, EI leads to a number of fragment ions providing more structural information. However, due to the extensive fragmentation of some ADs in the EI-mode, the positive chemical ionization mode (PICI) could provide more selectivity as this technique often gives molecular mass information. Negative chemical ionization (NICI) can improve sensitivity as compared to PICI or EI for the determination of compounds with electronegative moieties, either present in their original structure or obtained after derivatisation [33,34].

LC–MS methods have the great advantage that no derivatisation is needed, leading to a shorter sample preparation time and thus higher-throughput. However, availability, high separation power and comparative low cost of the equipment still make GC–MS instruments very attractive in many laboratories.

This paper evaluates the performance of EI and CI (both PICI and NICI) in a GC–MS method for the simultaneous determination of new generation ADs and their active metabolites in plasma.

Although CI can offer advantages in selectivity and sensitivity, there has never been a GC–MS CI method published for monitoring these antidepressants. In this manuscript, the different ionization techniques are compared during the validation of this simultaneous determination procedure by GC–MS. This method is of interest for therapeutic drug monitoring (TDM) laboratories as it offers the analytical strategy for each of the individual antidepressants. In addition, the compounds selected are the highly prescribed new generation ADs in combination with their active metabolites. TDM of the metabolites can offer additional information on metabolic activity and compliance. Therefore, we validated the analytical procedures using three different ionization methods for measurement of the following compounds: mirtazapine, viloxazine, venlafaxine, trazodone, citalopram, mianserin, reboxetine, fluoxetine, fluvoxamine, sertraline, maprotiline, melitracen, paroxetine, desmethylfluoxetine, desmethylmianserin, desmethyl-mirtazapine, desmethylsertraline, desmethylmaprotiline, desmethylcitalopram and didesmethylcitalopram.

2. Experimental

2.1. Reagents

2.1.1. Chemicals

Venlafaxine·HCl and *O*-desmethylvenlafaxine maleate (ODMV) were provided by Wyeth (New York, NY, USA). Organon (Oss, The Netherlands) donated mianserin·HCl, desmethylmianserin·HCl, mirtazapine, and desmethylmirtazapine maleate, while sertraline·HCl, desmethylsertraline maleate, and reboxetine methanesulphonate were a gift from Pfizer (Groton, CT, USA). Lundbeck (Valby, Denmark) offered citalopram.HBr, desmethylcitalopram·HCl, didesmethylcitalopram tartrate hydrate (DDMC), and melitracen·HCl. ACRAF (Roma, Italy) provided trazodone·HCl and its metabolite *m*-chlorophenylpiperazine·HCl, whereas paroxetine·HCl hemihydrate was donated by GlaxoSmithKline (Erembodegem, Belgium) and viloxazine·HCl by AstraZeneca (Brussels, Belgium). Fluvoxamine maleate and maprotiline·HCl were provided by Solvay Pharmaceuticals (Weesp, The Netherlands) and Novartis Pharma (Basel, Switzerland), respectively. Fluoxetine·HCl, desmethylfluoxetine·HCl and 1-(heptafluorobutyryl) imidazole (HFBI) were purchased from Sigma–Aldrich (Steinheim, Germany). Promochem (Molsheim, France) delivered fluoxetine-d_6 oxalate, mianserin-d_3, maprotiline-d_3 and paroxetine-d_6 maleate (100 μg/ml in MeOH). The following reagents were purchased from Merck (Darmstadt, Germany): ammonia-solution 25%, orthophosphoric acid (85%), sodium dihydrogen phosphate monohydrate, methanol and water (HPLC grade), and toluene (Suprasolv). The strong cation exchanger (Strata SCX with 200 mg sorbent mass) was obtained from Phenomenex (Bester, Amstelveen, The Netherlands). Vials, glass inserts and viton crimp-caps were purchased from Agilent technologies (Avondale, PA, USA). Drug-free EDTA plasma was obtained from healthy volunteers and harvested within 2 h after a 10-min centrifugation period at 1200 *g*.

2.1.2. Preparation of standard solutions and calibrators

Primary stock solutions of each individual AD were prepared in methanol at a concentration of 1 mg/ml and stored at −20 °C. A standard mixture was obtained by mixing these individual primary stock solutions and by further diluting with methanol until a concentration of 0.05–0.125 mg/ml, depending on the therapeutic range of the compound. After preparation, it was stored protected from light at approximately −20 °C. Further