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Separation and determination of chlorophenylpiperazine isomers in confiscated pills by capillary electrophoresis

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ABSTRACT

A simple capillary electrophoretic method with spectrophotometric UV detection at 236 nm has been developed for the selective separation and determination of 1-(2-chlorophenyl)piperazine (oCPP), 1-(3-chlorophenyl)piperazine (mCPP) and 1-(4-chlorophenyl)piperazine (pCPP) in confiscated pills. Several cyclodextrin derivatives were tested to compose the background electrolyte (BGE). The optimized BGE contained 20 mmol/L phosphoric acid adjusted to pH 2.5 with triethylamine and 10 mmol/L α-cyclodextrin, which provided acceptable resolution of analytes and candidate interferents in less than 15 min. The analyses were performed at constant voltage of 25 kV in 60 cm (effective length 50 cm; 50 μm i.d.) uncoated fused-silica capillary maintained at 25 °C with sample injection at 4826 Pa for 8 s. Procaine at a concentration of 0.1 mg/mL was used as internal standard (IS). Possible interference from other drugs such as amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine, 3,4-methylenedioxymethamphetamine, 3,4-methylenedioxy-N-ethylamphetamine, 1-(3-trifluoromethylphenyl)piperazine and cocaine was also examined. The analytical curves were linear (R^2 = 0.9994–0.9995) in the range of 10–200 μg/mL (for oCPP and mCPP) and 20–200 μg/mL for pCPP. Limits of detection (LODs) were 2.0 μg/mL (oCPP), 2.5 μg/mL (mCPP) and 3.5 μg/mL (pCPP). Intraday precision at three concentration levels and six replicates of each level (10, 100, 200 μg/mL of each analyte; n = 18) was evaluated for the corrected peak area ratio of analyte to IS and the migration times giving RSDs ≤ 4.9%. The accuracy was estimated for mCPP by a recovery test at the same three concentration levels and recoveries varied from 101.0 to 101.6%. The method has been successively applied to the analysis of 17 confiscated pills based mostly on mCPP.

1. Introduction

Chlorophenylpiperazines belong to a relatively new class of piperazine designer drugs that appeared in the illicit market a few years ago [1,2]. According to the position of chlorine atom in the phenyl ring three positional isomers (*ortho*, *meta* and *para*) of chlorophenylpiperazine can be derived (Fig. 1). Structure–activity studies on the related phenylpiperazines revealed that the conformation of the molecule induced by substitution of the phenyl ring is crucial for selective reaction with the final receptors [3]. Although similar research has not been conducted with chlorophenylpiperazines, different behaviors of the individual CPP isomers can be expected. mCPP is an established 5HT receptor agonist whose activity is based on interaction with various serotonin receptors, as well as adrenergic and dopaminergic receptors, leading to secretion of serotonin. Only limited investigations on the properties of oCPP and pCPP have been made. Similarly to mCPP, pCPP has measurable serotonergic effects, although it seems to act as a non-selective agonist. On the other hand, it has been demonstrated that oCPP is an antagonist of the 5HT2C receptor, therefore, it is unlikely to produce similar effects to mCPP. Both mCPP and pCPP have been reported in illicit products and are controlled substances in many countries. Therefore, there is a need for methods to distinguish mCPP among its positional isomers in confiscated materials.

The 1-(3-chlorophenyl)piperazine (mCPP), originally known as a serotonine probe in psychiatric research or as the active metabolite of the antidepressant Trazodone, has been recently abused as

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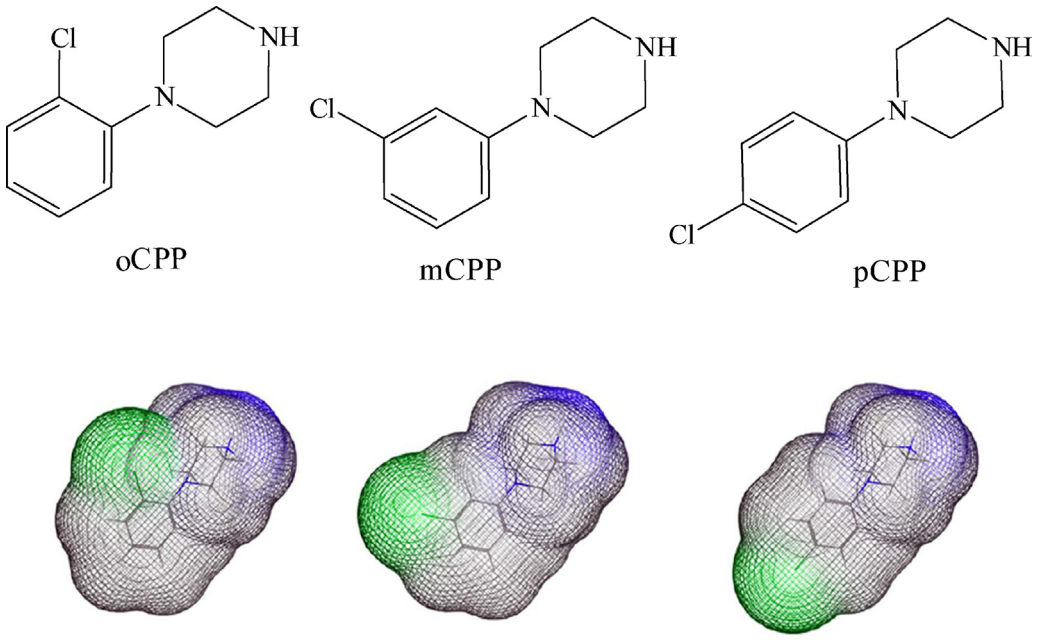

Fig. 1. Schematic representation including van der Waals surfaces of the chlorophenylpiperazine isomers under investigation in this work.

psychoactive substance in clandestine party pills [4]. The subjective experiences after mCPP ingestion are described to be similar to those of 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) of both negative (dysphoria, anxiety) and positive (euphoria) effects [5]. Moreover, mCPP pills are often designed to resemble those of ecstasy, and it can be found in combination with other piperazines and drugs of abuse (MDMA, cocaine, etc.) [6]. Although mCPP lacks neurotoxicity, many undesirable effects (migraine, nausea, severe hallucinations, panic attacks, etc.) associated with its ingestion [5,7] have tended to limit the use of mCPP as a recreational drug alternative to ecstasy [8].

The purpose of analysis of mCPP has changed in the literature: in early reports, mCPP has been inspected as an active metabolite of pharmaceuticals, whereas in more recent articles mCPP has been referred explicitly as a drug of abuse.

The analysis of mCPP in different biological matrices has been approached by a variety of chromatographic techniques. mCPP has been determined in rat plasma and brain by gas chromatography with nitrogen-phosphorus detection (GC-NPD) [9] and coupled with mass spectrometry (GC–MS) [10]. For the same biological samples, liquid chromatography using electrochemical (HPLC–ECD) [11], and ultraviolet (HPLC–UV) [12] detection has also been applied. Human plasma has been inspected for mCPP by HPLC–UV [13–17] and LC–MS [18]. The isomer has also been determined by HPLC–UV in human plasma, red blood cells [19] and plasma and breast milk [20]. All above mentioned methods were developed to give analytical support to mCPP activity research, pharmacokinetic studies and therapeutic drug monitoring.

Screening methods for the assay of designer drugs (amphetamines, tryptophans and/or piperazines, including mCPP) in body fluids involve GC–MS [21–23] and LC–MS [24]; hair samples were analyzed by GC/MS [25]. Methods related to confiscated drug material assays include LC–MS [26] and desorption atmospheric pressure photoionization-mass spectrometry (DAPPI-MS) [27].

The discrimination of the three chlorophenylpiperazine isomers in confiscated pills has only been demonstrated by two chiral methods based on HPLC–DAD [28] and capillary electrophoresis coupled to mass spectrometry, CE–MS/MS, in an unpublished work [29]. For the HPLC–DAD method, the separation was achieved within 14 min using a Chiracel OJ-RH column with a mobile phase composed of 30:70 (v/v) triethylamine:methanol, pH 9. The method was validated and tested for interferents such as amphetamine, MDMA and caffeine. LODs for all CPPs were in the order of 0.5 μg/mL. The CE–MS/MS method used a modified cyclodextrin (10 mmol/L HP-β-CD) as chiral selector in a volatile background electrolyte composed of 100 mmol/L formic acid and 10% 2-propanol, pH 2.4. Although resolution of the three CPP isomers was achieved in 13 min, the authors report difficulties due to the use of cyclodextrins with the MS detector and no validation data were provided to attest the method sensitivity and robustness.

The objective of this work was to develop and to validate a much simpler and reasonably fast capillary electrophoresis (CE) method employing diode array UV detection for the selective separation and determination of the three chlorophenylpiperazine isomers in illegal pills seized by Sao Paulo State Police. Several CD modifiers were devised to compose the background electrolyte and a few candidate interferents (drugs likely to be present as adulterants in the mCPP pills) were tested.

2. Materials and methods

2.1. Instrumentation

A capillary electrophoresis system model Proteomelab™ PA 800 from Beckman Coulter Inc. (Fullerton, CA, U.S.A.) coupled with diode array detector (DAD) was used for all analyses. Data acquisition and processing were performed with Beckman Coulter's 32 Karat™ software (version 7.0). The pH was measured with a DIGIMED pHmetro DM20 (Sao Paulo, Brazil) equipped with original glass electrode and calibration buffers (pH 4.10 and 6.86).

2.2. Capillary electrophoresis procedures

Separations were carried out in uncoated fused-silica capillaries of total length 60 cm (effective length 50 cm) and 50 μm inner diameter, purchased from ISB (BomPrincípio, RS, Brazil).

Before use, capillaries were conditioned with 1 mol/L NaOH for 30 min followed by ultra-pure water for 30 min at 144 790 Pa. At the