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TECHNICAL NOTE

Separation of positional CPP isomers by chiral HPLC-DAD of seized tablets

Jennifer Schürenkamp · Justus Beike · Heidi Pfeiffer · Helga Köhler

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Abstract *Meta*-chlorophenylpiperazine, one of the synthetic piperazine-derived designer drugs, is to date controlled as an illicit substance in five European member states. Depending on the position of the chlorine atom, different positional isomers of CPP (*ortho*-, *meta*- and *para*-) are possible. Therefore, there is a need to develop an analytical method for the separation and identification of the three 1-chlorophenylpiperazines in tablets containing CPP. In this work, the position isomers o-, m- and p-CPP were separated by liquid chromatography (HPLC) on a reversed-phase chiral column. Different mobile phase compositions and pH ranges were systematically studied to find optimum chromatographic conditions. Best results were achieved with isocratic mobile phase of triethyl amine buffer and methanol (*V*/*V*= 70/30) at pH 9 with a flow rate of 0.8 ml/min. The method was validated in terms of selectivity, linearity, limit of detection and quantification and precision. At last, the developed method was successfully applied on seized ecstasy tablets.

Keywords Chlorophenylpiperazine · m-CPP · Positional isomers · Chiral column · HPLC-DAD

Abbreviations

CPP	Chlorophenylpiperazine
o	*Ortho*
m	*Meta*
p	*Para*
MDMA	Methylendioxymethamphetamine
QC	Quality control
LOD	Limit of detection
LOQ	Limit of quantification
RSD	Relative standard deviation
S/N ratio	Signal-to-noise ratio

J. Schürenkamp (✉) · H. Pfeiffer · H. Köhler
Institute of Forensic Medicine Münster,
Röntgenstr. 23,
48149 Münster, Germany
e-mail: Jennifer.Schuerenkamp@ukmuenster.de

J. Beike
Institute of Forensic Medicine Cologne,
Melatengürtel 60-62,
50823 Cologne, Germany

Introduction

1-(3-Chlorophenyl)-piperazine is the systematic chemical name of a synthetic piperazine-derived designer drug that is better known by one of its codenames *meta*-chlorophenylpiperazine (m-CPP). m-CPP is known as an intermediate in the manufacture of trazodone and the related antidepressants nefazodone, etoperidone and mepiprazole. It also occurs as an active metabolite of those drugs. A further legitimate use of m-CPP is as a model reference compound in neurochemical studies such as prototypic serotonergic agonist [1, 2, 24].

m-CPP appears to have both 5-HT-releasing and post-synaptic agonist effects [3, 4]. Therefore a serotonergic syndrome may occur after m-CPP intake [6, 8], including anxiety, dizziness, confusion, shivering, sensitivity to light and noise, fear of losing control, migraine and panic attacks [5]. m-CPP shares a wide range of subjective effects with MDMA. In a study in which humans were trained to distinguish between D-amphetamine and m-CPP, half of the participants reported MDMA to be like amphetamine and half like m-CPP [7, 25]. Unlike MDMA, no cases of fatal poisoning by m-CPP have been reported so far but the increasing abuse of designer drug tablets with usually unknown composition increases the risk of severe intoxication and even fatal complications [9–11].

The first official notifications about m-CPP as a drug of abuse were received by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) and Europol in 2005.

Most illicit products containing m-CPP were tablets, usually marked with logos typical for ecstasy tablets, indicating that m-CPP is sold in the same user environment as ecstasy. Tablets have been known by a number of street names, e.g. regenboogies, arlequin, rainbow, rolls royce or smarties due to their multi-coloured inclusions or different logos. The amount of m-CPP in tablets varied substantially (8–80 mg) and often there are combinations with other psychoactive ingredients such as MDMA or other piperazines. Powders and capsules are less common.

Because of the commercial availability of m-CPP as a white powder or in solution, there was no need for illicit production. However, there were several routes to m-CPP synthesis, e.g. a reaction of the simple chemicals diethanolamine and 3-chloroaniline as precursors.

In the whole of Europe, there was a 10-fold increase in the number of seizures and amount of m-CPP in 2006 compared to 2005 [1, 2, 12, 13]. All three positional isomers of CPP are controlled substances in Greece and Belgium but in Denmark, Hungary, Lithuania and Germany, and only the m-CPP isomer is on the list of controlled substances.

In Germany, m-CPP has been a controlled substance since 14 February 2007 [13], and therefore, analytical methods are needed to identify m-CPP. The fact that there are three different positional isomers of CPP has to be taken care of. Depending on the position of the chlorine atom, possible CPP isomers are o-, m- and p-CPP. The molecular structures are shown in Fig. 1.

So far, only one analytical method has been described for the separation and identification of the three 1-chlorophenylpiperazines by CE-ESI-MS/MS [14].

In this work, the separation of the positional isomers has been achieved by simple instrumentation using isocratic liquid chromatography on a chiral column and diode array detection (chiral HPLC-DAD).

Experimental

Chemicals

o-CPP base and p-CPP hydrochloride were obtained in powder form from Sigma-Aldrich (Steinheim, Germany), whereas m-CPP hydrochloride was purchased from LGC (Wesel, Germany). All other chemicals were of analytical grade and obtained from different manufacturer: triethyl amine p.a. (Fluka, Germany), acetic acid p.a. (Merck, Germany), methanol pestiscan (LabScan, Poland) and acetonitrile super gradient (LabScan, Poland).

Instrumentation

Chromatography was performed with a Hewlett Packard (HP) Series 1100 HPLC-System equipped with a binary pump, autosampler, column oven and a DAD, and data were processed using chemstation software.

Chromatographic and detection conditions

Compounds were separated on a Chiracel® OJ-RH column from Diacel chemical industries with a length of 150 mm, a diameter of 4.6 mm and a particle size of 5 µm. The isocratic mobile phase was a 30:70 (*V*/*V*) mixture of triethyl amine buffer and methanol adjusted to pH 9 and with a flow rate of 0.8 ml/min. The column was maintained at 40°C, and the eluent was monitored at 254 nm. The injection volume was 10 µl.

Sample preparation

Confiscated powder and tablets were prepared by dissolving 40 mg in 20 ml methanol, and this solution was further diluted until the corresponding analyte signal was in the calibration range.

Assay validation

Preparation of solutions and QC samples

Methanolic stock solutions were prepared in concentrations of free bases: 1 mg/ml o-CPP, m-CPP and p-CPP. From the stock solutions, a methanolic spiking solution was prepared containing all analytes. All solutions were stored at −20°C.

Pools of QC samples containing CPP were prepared at three different concentrations: 2.5 µg CPP/ml (limit of quantification (LOQ)), 10 µg CPP/ml (medium QC) and 25 µg CPP/ml (high QC).

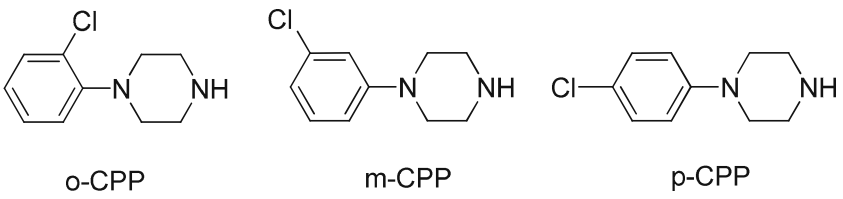


Fig. 1 Molecular structures of positional CPP isomers

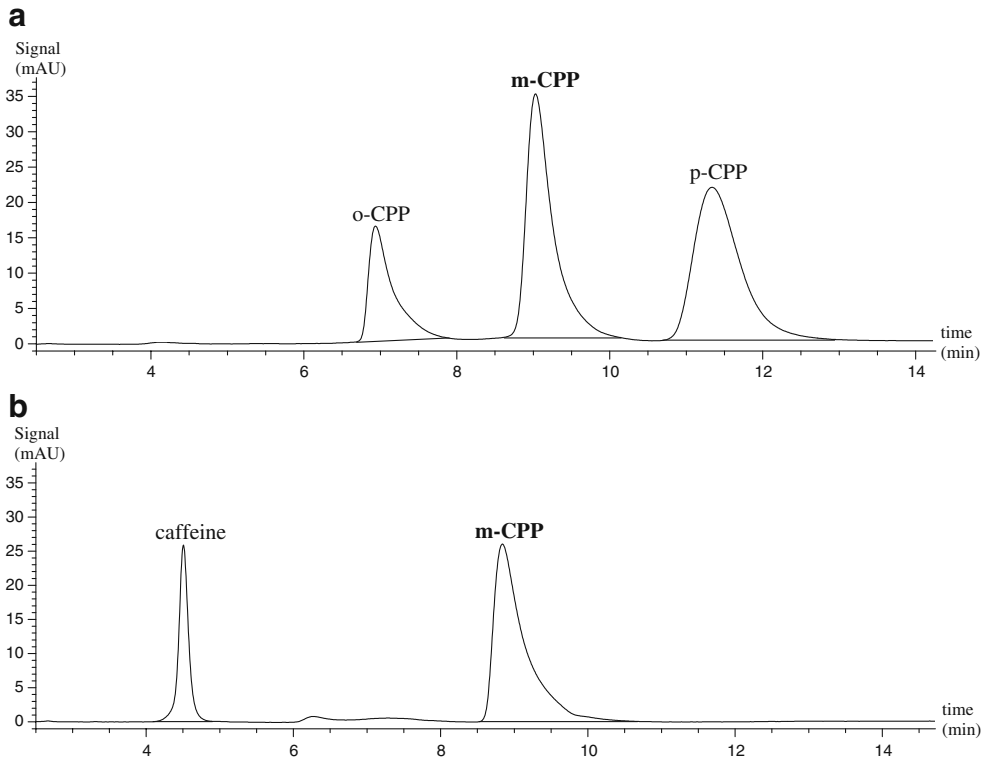


Fig. 2 **a** Chromatographic separation of positional CPP isomers. **b** Chromatogram of a confiscated sample ("bull-head") containing m-CPP and caffeine

Selectivity

Although a GC-MS analysis is done for identification of CPP, method selectivity has to be checked to ensure the absence of peaks that might interfere with the detection of the analytes. Therefore, different possible ingredients, e.g. amphetamine, MDMA or caffeine, were analysed.

Linearity of calibration and precision

Calibration standards at six different concentrations (2.5, 5, 10, 15, 20 and 25 µg CPP/ml methanol) were analysed in replicates (n = 6).

QC samples at three different concentrations (2.5, 10 and 25 µg CPP/ml) were analysed in duplicate after an 8-day interval.

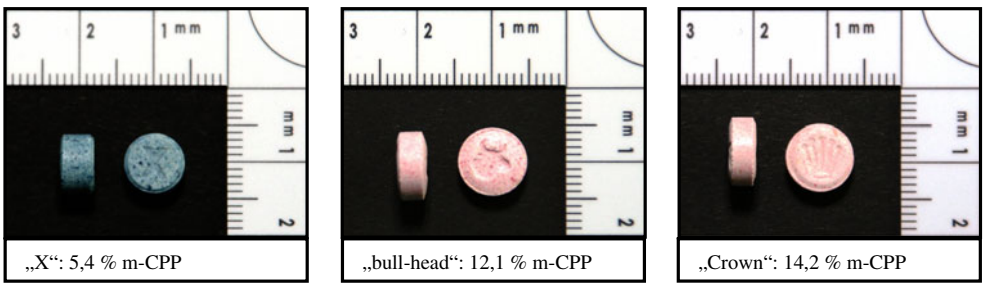
Limits

The lowest point of the calibration curve was the LOQ. It was tested whether the signal-to-noise ratios (S/N) of all analytes was greater than 10 and the precision was relative standard deviation (RSD) <20%. A signal-to-noise ratio greater than 3 was defined as the limit of detection (LOD).

Proof of applicability

Confiscated drugs were assayed with the described method.

Fig. 3 Confiscated drugs



Results and discussion

Analytical strategies in clinical and forensic toxicology are principally designed to start with relatively simple and quick screening procedures [15]. Therefore the use of a ubiquitous method such as GC-MS in scan mode is preferred in order to get as much information as possible. The mass-selective detection method can be used to identify CPP, but is not able to distinguish between the positional isomers. Because in four different countries only m-CPP is a controlled substance and not o- and p-CPP, there is demand for an analytical confirmation method that can detect o-, m-, p-CPP isomers.

Several analytical methods have been described for the identification of various amphetamines and analogues including m-CPP, among them GC and CE separations combined with mass-selective and UV detection [14, 16–21]. To this day, only one method has been described for distinguishing between the three positional isomers of CPP by chiral CE-ESI-MSMS [14]. For other piperazine isomers (o-/p-methoxyphenylpiperazine), a chiral CE-DAD method is used [19].

Chiral separation procedures are not only applicable for enantiomeric but also suitable for separating positional isomers. Instead of complex chiral CE separation, another possibility for positional isomer separation is chiral reversed-phase liquid chromatography [22] and was used in this study for the separation of the three CPP isomers.

The chiral column, Chiracel OJ-RH, has a packing composition of cellulose-tris-(4-methylbenzoat) coated on a 5 µm silica gel. It is mainly used for chiral separation of enantiomers because in addition to the usual separation results from the varying solubility of the substances between the stationary and mobile phase, there is separation according to the lock and key principle [23].

In order to achieve baseline separation without peak-tailing in short analysis times, different mobile phases with different pH ranges were used. It was observed that the best results (selectivity and resolution) were achieved with a mixture of methanol and triethyl amine (70/30) at pH 9 (see Fig. 2).

The validation data were evaluated by valistat® software. The calibration was linear, and the variances across the calibration range homogenous over the whole concentration range. Intra- and interprecision data, expressed as RSD were in the required ranges of ≤ 15% and ≤20%, respectively, at LOQ. Furthermore, the assay was found to be selective for m- and p-CPP because of different retentional behaviour compared to other common ingredients of ecstasy tablets. o-CPP co-elutes with MDE and MDMA, but has a different UV spectrum. The LOQ corresponded to the lowest concentration of the calibration (2.5 µg CPPs/ml) curve with a signal-to-noise ratio of at least 10. A sample containing 0.5 CPPs µg/ml methanol was still detectable with S/N>3 (LOD).

Analysis of confiscated tablets (Fig. 3) by GC-MS in scan mode resulted in the identification of chlorophenylpiperazine. In addition, the tablets "bull-head" contained caffeine. The chiral reversed-phase diode array detection revealed that only the regulated m-CPP and not the legal o-/p-CPP were ingredients of the tablets. The m-CPP yield of the tablets was between 5.4% and 14.2%.

Conclusions

In this work, it was found that the use of chiral reversed-phase columns for liquid chromatography with diode array detection is a simple method for distinguishing between the different positional isomers *ortho*-, *meta*- and *para*-chlorophenylpiperazine. The developed assay was successfully applied for the identification of the positional isomers of CPP preparations.

A simple reversed-phase chiral HPLC method has been developed for the separation of the positional isomers *ortho*-, *meta*- and *para*-chlorophenylpiperazine and has been validated for forensic application.

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