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Regioisomeric and enantiomeric analyses of 24 designer cathinones and phenethylamines using ultra high performance liquid chromatography and capillary electrophoresis with added cyclodextrins

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ABSTRACT

Designer: phenethylamines (PEAs) and cathinones have been encountered worldwide. Complete characterization of these substances can be challenging due to their chirality and variably substituted phenyl rings. In this study, 24 PEAs and cathinones were analyzed by ultra high performance liquid chromatography with photo diode array detection (UHPLC-PDA) on a variety of stationary phases, and by capillary electrophoresis on a dynamically coated capillary with PDA detection (CE-PDA). In the UHPLC-PDA study, a BEH Phenyl column resolved 18 of the 24 regioisomers in 8 min, with good discrimination of the PEAs. In contrast, capillary zone electrophoresis (CZE) on a dynamically coated capillary partially or baseline resolved only 10 of the 24 regioisomers, but with improved discrimination of mono-substituted cathinones. A second series of CE-PDA experiments using 80 mM (2-hydroxypropyl)-β-cyclodextrin (HP-β-CD) in the run buffer resolved all 24 regioisomers and all but two sets of enantiomers within 18 min. Five illicit samples were successfully analyzed using the described methods.

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1. Introduction

Novel "designer" drugs, including a wide variety of phenethylamines and cathinones, have continuously emerged in the recreational drug market over the past 10 years. Phenethylamines are substances structurally related to phenethylamine or amphetamine; whereas, cathinones are β-keto-phenethylamines structurally related to cathinone or other naturally occurring alkaloids [1–5].

In order to evade controlled substance statutes–which are usually written very specifically with respect to the substance under control–"designer" phenethylamines and cathinones are typically crafted by replacing phenyl ring hydrogens with various substituents. The most common single substituents include short alkyl chains such as methyl or ethyl, short alkoxy chains such as methoxy and ethoxy, and halogens such as fluoro or chloro. For each such substituent, three ring positional isomers (*ortho*, *meta*, or *para*) are possible. Multiple substituents (e.g., methylenedioxy-, dimethoxy-, dimethyl-, etc.) further expand the possibilities.

Most phenethylamines and cathinones are also chiral; of the 24 isomers analyzed herein (Fig. 1), only 5,6-methylenedioxy-2-aminoindane (5,6-MDAI) is achiral. Although there is only limited pharmacological data available on these drugs, the enantiomers would be expected to have significantly different pharmacological effects and potencies. For example, the (−)-cathinone and (+)-amphetamine forms exhibit considerably stronger CNS stimulant activities versus their enantiomers [6,7].

Complete characterization of these drugs requires determination of both the regioisomer and the enantiomer. Furthermore, this information can also help establish the synthetic routes used for their manufacture.

Ring positional isomers usually display easily distinguishable nuclear magnetic resonance (NMR) and infrared (IR) spectra;

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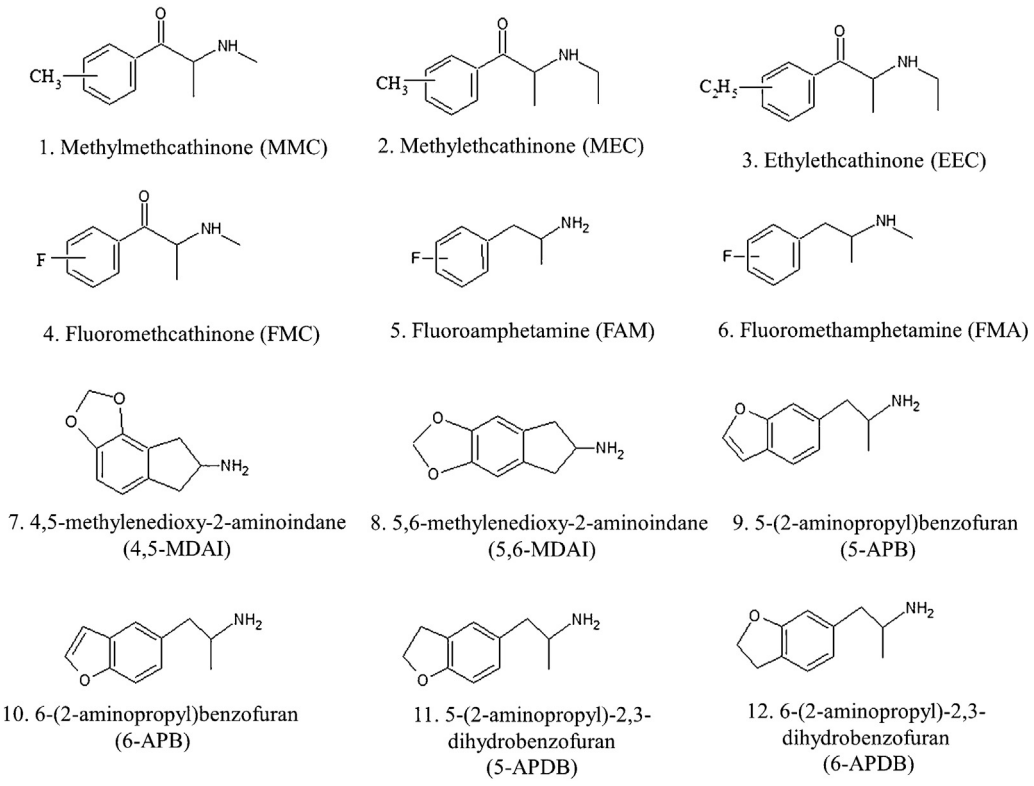

Fig. 1. Chemical structures of cathinone and phenethylamine derivatives used in this study.

however, their mass spectra are usually highly similar or virtually identical, particularly the *meta*- and *para*-isomers [8–13]. Differentiation of mono- or di-substituted phenethylamines and cathinones has been previously accomplished using GC/MS or GC-MS/MS, but only where the isomers were distinguishable by GC [8,14,15]. The three positional isomers of methylmethcathinone (MMC) were fully resolved by employing an HP ULTRA1 (a non-polar column) [8].

Direct analysis requires acid/base workup; however, many cathinones undergo degradation under alkaline conditions, or break down on heated injection ports [8,15–17], and many *meta*- and *para*-isomers are only poorly resolved by GC [8,11,18]. Derivatization can improve GC performance; however, it is more time consuming. Due to these limitations, GC, GC/MS, and related methods are not well suited for general analysis of these type compounds.

Liquid phase separations (notably HPLC and CE) have also been utilized for forensic analyses of basic drugs. We previously reported a UHPLC-PDA/MS method for screening of emerging drugs [19]. Using this method, the *ortho*-, *meta*-, and *para*-isomers of fluoroamphetamine (FA), fluoromethamphetamine (FMA), and fluoromethcathinone (FMC) were baseline separated; however, the method was unable to discriminate between 3- and 4-methylethcathinone (MEC).

Due to its low solvent consumption, easy setup and operation, and alternate separation mechanism versus HPLC, CE with chiral additives is well-suited for chiral separations [20,21]. In addition, dynamically coated capillaries runs allows for shorter analysis times and improved migration time reproducibility [22,23].

Enantiomeric separations can be achieved by specialized GC, HPLC, CE, and supercritical fluid chromatography, including the use of chiral stationary phases, or an achiral column with a chiral mobile phase, or (more traditionally) following pre-column derivatization with an optically pure chiral reagent to create diastereomers [24–33]. However, these techniques are typically highly compound selective and usually require a trial-and-error process, which can be both time consuming and expensive.

HPLC using cyclodextrin (CD) as a chiral additive to the mobile phase has been reported for chiral separation of phenethylamines and cathinones [33,34]; however, these methods suffered from low chromatographic efficiency and significantly reduced column life. In contrast, CE with the addition of chiral CDs has been successfully employed for analyses of various drugs, including amphetamines and cathinones [35,36,22,37–40].

In this study, regioisomeric and chiral separations of 24 phenethylamines and cathinones were conducted using achiral and chiral UHPLC and CE. Regioisomeric analyses were investigated by using UHPLC-PDA and CZE-PDA with dynamically coated capillaries, while enantiomeric analyses were conducted using several chiral CDs. To our knowledge, this is the first report of the use of a chiral additive in the mobile phase for regioisomeric separations using UHPLC.

2. Materials and methods

2.1. Chemicals and reagents

All drug standards were obtained from the reference collection of this laboratory. Puriss p.a. grade formic acid (≥98%); HPLC grade phosphoric acid; 85%; β-cyclodextrin (β-CD, FW 1,135.01); heptakis (2,6-di-O-methyl)-β-cyclodextrin (DM-β-CD, FW 1,331.4); (2-hydroxypropyl)-β-cyclodextrin (HP-β-CD, average mw 1380); and reagent grade sodium hydroxide were all purchased from Sigma Aldrich (St. Louis, MO). HPLC grade methanol and acetonitrile were obtained from Burdick and Jackson (Muskegon, MI). High-purity, deionized (D.I.) water was obtained from a PURELAB Ultra Mk2 (ELGA LabWater Global Operations, UK). CElixir initiator solution A, CElixir initiator solution B, and 0.1 N sodium hydroxide were purchased from Microsolv (Eatontown, NJ).