Chiral and Stable Isotope Analysis of Synthetic Cathinones

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Abstract

In the past decade synthetic cathinones have appeared in drug markets worldwide. Chiral analysis can provide information on relative enantiomeric abundances of synthetic cathinones in their drug products, potentially giving a signature of these products and hence linking the products or excluding them from possible sources. Additionally, due to natural variations of relative stable isotopic abundances of elements, stable isotope analysis of synthetic cathinone drug products provides the stable isotopic signature of the products and hence also has potential to provide additional information for the purpose of drug intelligence. Therefore, both molecular (chirality) and physicochemical (relative stable isotopic abundances) properties are important for forensic chemists to investigate. Chiral and isotope ratio mass spectrometric analysis are becoming increasingly important to forensic chemists and therefore this review will focus on an overview of these techniques applied to the synthetic cathinones.

Keywords

Capillary electrophoresis; chiral analysis; drug intelligence; forensic chemistry; gas chromatography; high performance liquid chromatography; isotope ratio mass spectrometry; novel psychoactive substance; stable isotope analysis; synthetic cathinone

Abbreviations

(−)-MTPA, (*S*)-(−)-α-Methoxy-α-(trifluoromethyl)phenyl acetic acid/(−)-Mosher's acid; (+)-18-C-6-TCA, (+)-(18-Crown-6)-2,3,11,12-tetracarboxylic acid; (+)-MTPA, (*R*)-(+)-α-Methoxy-α-(trifluoromethyl)phenyl acetic acid/(+)-Mosher's acid; (*R*)-(−)-MTPA-Cl, (*R*)-(−)-α-Methoxy-α-(trifluoromethyl)phenylacetyl chloride/(*R*)-(−)-Mosher's acid chloride; 3,4-MDMC, 3,4-Methylenedioxymethcathinone; 3,4-MDPV, 3,4-Methylenedioxypyrovalerone; 4-CMC, 4-Chloromethcathinone; 4-MMC, 4-Methylmethcathinone; CD, Cyclodextrin; CE, Capillary electrophoresis; CEC, Capillary electrochromatography; CM-β-CD, Carboxymethyl-β-cyclodextrin; DAD, Diode array detection; DCC, *N*,*N*-Dicyclohexylcarbodiimide; DL-4662, 1-(3,4-Dimethoxyphenyl)-2-(ethylamino)pentan-1-one; DM-β-CD, Heptakis(2,6-di-*O*-methyl)-β-cyclodextrin; FID, Flame ionisation detection; GC, Gas chromatography; HPLC, High performance liquid chromatography; HP-β-CD, (2-Hydroxypropyl)-β-cyclodextrin; HS-γ-CD, Highly sulfated-γ-cyclodextrin; IUPAC, International Union of Pure and Applied Chemistry; LIF, Laser-induced fluorescence spectroscopy; *L*-TPC, (*S*)-(−)-*N*-(Trifluoroacetyl)prolyl chloride; MS, Mass spectrometry; NBD-F, 4-Fluoro-7-nitrobenzofurazane; NMR, Nuclear magnetic resonance spectroscopy; NPS, Novel psychoactive substance(s); R_s, Resolution factor(s); SBE-β-CD, Sulfobutyl ether-β-cyclodextrin; SFC, Supercritical fluid chromatography; S-β-CD, Sulfated-β-cyclodextrin; UV/VIS, Ultraviolet/Visible spectroscopy; α, Separation factor(s)

Contents

1 Introduction

Novel Psychoactive Substance(s) (NPS) are compounds of concern to law enforcement agencies, governments and society. According to the European Monitoring Centre for Drugs and Drug Addiction, synthetic cathinones are the second largest group of NPS, accounting for seventeen percent of all NPS seizures in 2014 [1]. This group of NPS were first detected in Europe in 2004 and since then, one hundred and three new derivatives have been identified. Being the β-keto analogs of amphetamine type compounds (Fig. 1), they are sold in local head shops and on the Internet as 'legal' replacements for traditional stimulant drugs of abuse. In 2014, greater than eight thousand seizures weighing more than a tonne were made across Europe. Synthetic cathinones may be controlled in the UK within the Misuse of Drugs Act (1971) and the Psychoactive Substances Act (2016), with other countries having similar legislation in place.

Since the explosion of synthetic cathinones, many analytical methods have been developed for both chemical and toxicological analysis. Methods were developed to focus on identification of bulk drug products seized by law enforcement agencies and quantification of the drugs in biological specimens. In 2012, a review was published which contained a comprehensive discussion on mass spectral interpretation of synthetic cathinones obtained from electron ionisation and electrospray ionisation–collision induced dissociation (under quadrupole time of flight mass analyser) [2]. This approach can aid identification of these compounds in 'legal high' products by using common mass spectrometric techniques in a forensic laboratory. Last year, a mini-review discussed a variety of techniques applied to synthetic cathinone analysis [3]. These included presumptive colour test and immunoassay and other instrumental techniques such as surface enhanced Raman spectroscopy, ion mobility spectrometry, cyclic voltammetry, chromatography and mass spectrometry (MS). This review provided an insight into how some analytical techniques can be applied to identification and quantification of these compounds. Furthermore, in 2015 a review was published covering chromatography–mass spectrometry techniques used for analysis of synthetic cathinones in biological specimens [4].

From an analytical point of view, most literature methods deal with achiral analysis, which is fit for purpose in certain situations, but other circumstances may dictate a need for stereochemical information. For example, the synthesis of homochiral synthetic cathinones is possible (Fig. 2 (a) and (b)) [5-7] and it was found that keto-enol tautomerism of synthetic cathinones could happen under basic condition [6, 8-10], meaning that identification of an individual enantiomer and/or determination of relative amount of an enantiomeric pair could be important and useful to forensic chemists. This additional information can be obtained to trace which sources the drug products could possibly be linked to or excluded from, if they are specifically prepared under certain poorly controlled conditions but isolated as a stable hydrochloride or hydrobromide salt in a clandestine laboratory. A study has shown that enantiomeric ratios of synthetic cathinones in different drug products could be different [11]. On the other hand, depending on the actual synthetic cathinone, different enantioselective pharmacodynamics has been observed. While *S*-(−)-cathinone and *S*-(−)-methcathinone are about three-fold more potent than their *R* forms [12, 13], *R*-(+)-4-methylmethcathinone has stronger dopaminergic stimulating effects that *S*-(−)-4-methylmethcathinone [14]. Therefore, chiral analysis of synthetic cathinones is also important in toxicological and pharmacological studies.

From a synthetic perspective, this group of NPS are mainly produced by bromination of alkylphenylalkanone followed by reacting the intermediate with alkylamines (Fig. 2 (c)) [15-19]. In this situation, standard chemical techniques and chiral profiling sometimes would not provide the required level of information on possible sources. Therefore, other physicochemical properties should be taken into observation in addition to chirality. Stable isotope analysis has the potential to be helpful at this point for the following reasons:

(1) there are natural variations of relative stable isotopic abundances of the elements in the starting and reacting materials, cutting reagents and other chemicals added to the final drug products;

(2) there is variability among the parameters of drug synthesis between clandestine laboratories e.g. reaction routes and conditions, storage conditions of chemicals.

All of these could cause distinguishable variations of relative stable isotopic abundances of different elements in the drug products and hence stable isotopic signature of the products provides additional information about their possible sources to forensic chemists in a manner similar to enantiomeric ratios.

In this article, different methods of chiral analysis and existing stable isotope data of synthetic cathinones are reviewed in order to have an insight on how these two analytical techniques could be applied to forensic analysis of this important group of NPS.

2 Discussion

2.1 Chiral Analysis of Synthetic Cathinones

The three most common separation techniques namely, capillary electrophoresis, gas chromatography and high performance liquid chromatography, are used for chiral analysis of synthetic cathinones. In most cases except gas chromatographic analysis, ultraviolet (UV) detection was used because coupling with a more specific analyser–detector, mass spectrometer, is not necessary as enantiomers give identical mass spectra. Table 1–3 summarises the publications involving each of these separation techniques. Other techniques including capillary electrochromatography (CEC) are summarised in Table 4. In this section, we are going to discuss how these techniques have been applied to synthetic cathinones, including data relevant to chiral separation such as resolution factor (R_s). Note that baseline separation of a pair of enantiomers is indicated by $R_s = 1.5$.

2.1.1 Capillary Electrophoresis (CE)

CE has been broadly used for chiral analysis with different substituted cyclodextrins (CDs) as chiral selectors (Fig. 3). Sulfobutyl ether-β-CD (SBE-β-CD) was used for chiral separation of four synthetic cathinones [20]. While 4-methoxymethcathinone ($R_s = 1.17$) and 4-fluoro-α-pyrrolidinoheptiophenone ($R_s = 1.10$) enantiomers were partially separated, 3-methoxymethcathinone ($R_s = 2.31$) and 4-methoxy-α-pyrrolidinopentiophenone ($R_s = 2.41$) enantiomers were baseline separated. In addition to SBE-β-CD, heptakis(2,6-di-*O*-methyl)-β-CD (DM-β-CD) was applied to chiral separation of cathinone and methcathinone but different elution orders were shown [21]. While SBE-β-CD showed higher resolution for chiral separation of methcathinone ($R_s = 3.6$) than DM-β-CD ($R_s = 1.0$), mixing the two β-CDs worsened the separation ($R_s = 2.2$). Mixing two β-CDs was not successful as indicated in another study where DM-β-CD and (2-hydroxypropyl)-β-CD (HP-β-CD) were used [22].

This could be because of the complementary behaviour of the two chosen β-CDs. In the study, it was observed that for chiral separation of a range of synthetic cathinones (2-, 3-, 4-regioisomers of methylmethcathinone, methylethcathinone and ethylethcathinone), these two β-CDs were found to be complementary on their chiral selectivities. Interestingly, another two CDs highly sulfated-γ-CD (HS-γ-CD) and native β-CD were also found to give complementary chiral separation for some synthetic cathinones [23]. Native β-CD did not separate 4-methoxymethcathinone and 4-fluoromethcathinone enantiomers but these were separated using HS-γ-CD, while HS-γ-CD did not separate *N*,*N*-dimethylcathinone, ethcathinone, pentedrone, and buphedrone enantiomers but these were separated by native β-CD. In this study, the use of only HS-γ-CD as the selector with high resolution mass spectrometric detection was successfully applied to chiral analysis of synthetic cathinones in seized drug samples, allowing enantiomers of 3,4-dimethylmethcathinone and 3,4-methylenedioxymethcathinone (3,4-MDMC) to be identified. HS-γ-CD was also applied to chiral separation of methcathinone after labelling with a fluorescence dye 4-fluoro-7-nitrobenzofurazane (NBD-F), achieving baseline separation for this derivative [24]. In addition, combining HS-γ-CD with (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid ((+)-18-C-6-TCA) improved resolutions of chiral separation of six synthetic cathinones [25]. (+)-18-C-6-TCA was then used by the same research group for chiral separation of two primary amine synthetic cathinones, cathinone and 4-methylcathinone, achieving baseline separation [26]. This selector was applied in both conventional bench-top and portable ultrafast CE in the study, allowing the possibility of crime scene detection of the enantiomers. Another selector, sulfated-β-CD (S-β-CD), was applied to the chiral separation of 4-chloromethcathinone (4-CMC) with baseline separation almost achieved [27]. In a previous study conducted by the same research group, ten out of nineteen pairs of different synthetic cathinone enantiomers were baseline separated by this selector [28]. In this study, five CDs including native β-CD and S-β-CD were compared based on their chiral selectivities on three model synthetic cathinones (4-methylmethcathinone, butylone and naphyrone). Enantiomers of all three were not well separated by native β-CD but the resolutions were improved using S-β-CD (R_s = 1.3, 1.7 and 6.4 respectively). In contrast, a recent study showed that S-β-CD did not work as expected but native β-CD enabled the chiral separation of 4-methylmethcathinone (4-MMC) and 3,4-methylenedioxypyrovalerone (3,4-MDPV) [29], possibly because of the different experimental conditions applied such as the identity and pH value of buffer solution.

2.1.2 Gas chromatography (GC)

For chiral separation of synthetic cathinones by GC, a chiral stationary phase (Rt-βDEXsm) was used to separate cathinone enantiomers but poor resolution was observed [30]. Instead, the use of chiral derivatisation seems to be preferred by analysts and good resolution can be achieved. Although various derivatisation reagents have been employed (Table 2), (*S*)-(−)-*N*-(trifluoroacetyl)prolyl chloride (*L*-TPC) and α-methoxy-α-(trifluoromethyl)phenyl acetic acid/Mosher's acid (MTPA) anhydride were mostly used for the purpose. Both reagents react with synthetic cathinones on their amino groups to form amido derivatives (Fig. 4). Separation of the resultant diastereomers was commonly carried out by a general 5% diphenyl-95% dimethyl polysiloxane phase such as HP-5MS or DB-5.

Summarising three studies conducted by the same research group, twelve pairs of synthetic cathinone-*L*-TPC diastereomers were baseline separated (R_s = 1.52 – 5.7) on HP-5MS (30 m) column and ten pairs were partially separated (R_s = 0.5 – 1.4), although buphedrone-, butylone-, 3-methylmethcathinone- and 4-chloromethcathinone-*L*-TPC diastereomers were

separated under different temperature programs [10, 27, 31]. However, chiral separation of six of the experimented synthetic cathinones, including four cyclic tertiary amine members, was not successful since *L*-TPC cannot react with tertiary amine to form amide. In a recent study from another research group, using a longer column (60 m) with the same effective temperature program, only four out of thirty pairs of secondary amine synthetic cathinone-*L*-TPC diastereomers were not baseline separated (R_s = 1.04 – 1.42) [32]. Expectedly, resolutions of all common diastereomeric pairs between the two group's studies were improved by using a longer column but buphedrone- and 3-fluoromethcathinone-*L*-TPC diastereomers remained partially resolved. Being the sole experimented pair of primary amine synthetic cathinone-*L*-TPC diastereomers in the latter group's study, 4-methylcathinone-*L*-TPC diastereomers were greatly resolved (R_s = 14.89) compared to the best resolved pair, 4-methoxymethcathinone-*L*-TPC diastereomers (R_s = 8.92), among the thirty secondary amine synthetic cathinone-*L*-TPC diastereomers. Before the explosion of synthetic cathinones in drug markets, *L*-TPC was used successfully for chiral derivatisation of cathinone and phenalkylamines [33, 34] and methcathinone [5]. In the former two studies, MTPA anhydride was also used and has been applied to analysis of real samples, i.e. khat plant materials [33] and over-the-counter cold remedies [34]. The anhydride form of MTPA was prepared in situ by a reaction between *N*,*N*-dicyclohexylcarbodiimide (DCC) and the original acid (Fig. 4). MTPA anhydride has also been used to derivatise methcathinone [6] and 1-(3,4-dimethoxyphenyl)-2-(ethylamino)pentan-1-one (DL-4662) [35]. On HP-5MS column, the separation of DL-4662-MTPA diastereomers remarkably achieved a very high resolution (R_s = 19.35).

2.1.3 High Performance Liquid Chromatography (HPLC)

More than twenty years ago chiral derivatisation was carried out for separation of cathinone enantiomers using HPLC with an achiral stationary phase [36]. However, chiral separation of synthetic cathinones by HPLC later relied mainly on chiral stationary phases so that derivatisation is not necessary.

A research group bonded (+)-18-C-6-TCA covalently to aminopropyl and 3-(*N*-methylamino)propyl silica gel for chiral separation of twelve primary amine synthetic cathinones [37]. Except for cathinone enantiomers on the former phase (R_s = 1.47), baseline separation was achieved for all the other enantiomeric pairs on both phases (R_s = 1.89 – 3.87 and 1.50 – 7.17 respectively). The latter phase gives higher resolutions in general since amino hydrogen atoms on aminopropyl tethers, which are absent on 3-(*N*-methylamino)propyl tethers, form intramolecular H-bond with oxygen atoms on crown ether ring in the former phase and hence hindering that interaction between synthetic cathinones and the ring [38]. After noticing dynamically coated (3,3'-diphenyl-1,1'-binaphthyl)-20-crown-6 phase (Crownpak CR (+)) achieved a very high resolution (R_s = 16.96) for chiral separation of cathinone [39] but has a potential of leaching out from the column caused by running mobile phase, the research group applied covalently linked (3,3'-diphenyl-1,1'-binaphthyl)-20-crown-6 phase for chiral separation of the twelve primary amine synthetic cathinones [40]. Baseline separation was achieved for all of the enantiomeric pairs and the resolutions (R_s = 2.6 – 11.1) were improved in general compared to using the two (+)-18-C-6-TCA phases. The research group then modified this phase by treating it with *n*-octyltriethoxysilane, the aim being to protect the residual silanol groups of silica solid support and to increase lipophilicity of the phase using *n*-octyl group (Fig. 5 (a)) [41]. Resolutions of chiral separation of the twelve synthetic cathinones (R_s = 6.49 – 19.64) were significantly improved.

These results indicate that crown ether phases are good choices for chiral separation of primary amine synthetic cathinones.

An alternative to using a crown ether phase is a polysaccharide phase such as amylose tris [(*S*)-α-methylbenzylcarbamate] (Chiralpak AS-H) (Fig. 5 (b)) [35, 42]. Looking at two studies conducted by a research group, sixteen out of twenty-five pairs of synthetic cathinone enantiomers were baseline separated although chiral separation of DL-4662 was carried out on a longer column. All four cyclic tertiary amine synthetic cathinones (3,4-methylenedioxypyrovalerone, α-pyrrolidinopropiophenone, 4-methyl-α-pyrrolidinopropiophenon and naphyrone) were not enantio-separated and enantiomers of another tertiary amine member *N*,*N*-dimethylbutylone were poorly resolved (R_s = 0.5). This suggests H-bond involving the amino hydrogen atoms on synthetic cathinones could be playing an important role in the chiral recognition mechanism of this stationary phase and hence this phase might only be applicable to chiral separation of primary and secondary amine synthetic cathinones. This finding is also consistent with other studies that polysaccharide phases (Chiralcel OJ-H [9], Chiralcel OJ [43]) do not resolve tertiary amine synthetic cathinone enantiomers well. Recently, another research group also used the same stationary and mobile phases for chiral analysis of synthetic cathinones in 'legal high' products [11]. Baseline separation was achieved for all eight pairs of secondary amine synthetic cathinone enantiomers identified in the products while 3,4-methylenedioxypyrovalerone was not enantio-separated by this phase and agreed well with the other studies. Furthermore, this study also pointed out that 3,4-methylenedioxypyrovalerone was neither enantio-separated by Pirkle-type phases ((*S*,*S*)-Whelk-O 1 and *L*-Phenylglycine) nor by a teicoplanin based macrocyclic glycopeptide phase (Chirobiotic T) but it was successfully enantio-separated on two other homemade polysaccharide phases, amylose tris-3,5-dimethylphenylcarbamate and amylose tris-3,5-dimethoxyphenylcarbamate, with baseline separation (R_s = 3.11) observed from using the former one.

Chiral ion exchange type phases have been investigated in the past year [44] and chemical structures of the applied phase moieties are shown in Fig. 5 (c). Comparing structural differences among the three experimented phases with various mobile phase compositions, it was found that the performance of some interactions (e.g. H-bond and π-π) is strongly influenced by the size of a solvation shell around polarisable units of the stationary phases and analytes. For chiral separation of fourteen secondary amine synthetic cathinones, the best resolving phase among the three was found to be the syringic acid based one (R_s = 0.7 – 1.86). However, only four out of the fourteen enantiomeric pairs were baseline separated (R_s = 1.78, 1.84, 1.84 and 1.86), suggesting further investigation may be required. Other phases such as isopropyl-carbamate cyclofructan 6 (Larihc CF6-P) and vancomycin-based macrocyclic glycopeptide (Chirobiotic V2) were used for chiral separation of cathinone [45, 46] and methcathinone [45]. Although reasonable resolutions were achieved and individual enantiomers were quantified, further investigation is required for baseline separation and chiral separation of other novel synthetic cathinones.

2.1.4 Other Separation Methods

A capillary electrochromatographic method was developed for the chiral separation of ten synthetic cathinones [47]. The method was considered to be cheap and environmental friendly because of the needs of small quantities of chiral stationary phase chemicals and small volumes of reagents as comparing to that for HPLC. In the study, experimental conditions such as pH of mobile phases and concentrations of organic modifiers were

optimised and reasonable resolutions were achieved (R_s = 1.1 – 1.8) for chiral separation of the ten synthetic cathinones. However, only half of the experimented enantiomeric pairs were baseline separated. Following this study, seven synthetic cathinones (four in common) with a number of stationary phases were used to compare the chiral chromatographic performance of CEC directly with HPLC and supercritical fluid chromatography (SFC) [48]. Multiple stationary phases were required in each of the techniques for chiral separation of different synthetic cathinones. On comparison of the optimal resolutions among the techniques, HPLC was found to be the most applicable one. All experimented enantiomeric pairs were baseline separated under reverse or normal phase mode if different phases were used, with the exception being naphyrone enantiomers under normal phase condition (R_s = 1.2). On the other hand, however only three out of seven pairs were baseline separated using either CEC or SFC.

Apart from analytical separation, preparative separation of synthetic cathinone enantiomers was also demonstrated. Isolation of individual enantiomers is important because availability of single-enantiomers allows convenient analysis and easier understanding of the underlining chemical properties of individual enantiomers. It also helps to determine elution orders of the enantiomers in an analytical run. Pure 4-methylmethcathinone enantiomers were prepared in a relatively large scale (2 mg on column) by Whelk-O1 HPLC analytical column (250 mm × 4.6 mm I.D.) [49]. Pure 3,4-methylenedioxypyrovalerone enantiomers were prepared by amylose tris-3,5-dimethylphenylcarbamate phase HPLC semi-preparative column (200 mm × 7.0 mm I.D.) [11] and by co-crystallisation with (±)-2'-bromotartranilic acid [43]. In another study, enantioselective co-crystallisation of three synthetic cathinones (*N*,*N*-dimethylcathinone, *N*,*N*-diethylcathinone and α-pyrrolidinopropiophenone with three different tartaric acids ((+)-*O*,*O*'-di-*p*-toluoyl-*D*-tartaric, (+)-*O*,*O*'-dibenzoyl-*D*-tartaric and (−)-*O*,*O*'-dibenzoyl-*L*-tartaric acids) was possible [9]. However, racemisation during any of the isolation processes should be taken into consideration.

2.2 Stable Isotope Ratio Mass Spectrometric Analysis of Synthetic Cathinones

Over several decades, isotope ratio mass spectrometry has been used for distinguishing drugs including cannabis, cocaine, morphine, heroin, and amphetamine type stimulants [50, 51]. Due to the fact that NPS are emerging in drug markets, stable isotope analysis should not be limited to traditional drugs of abuse but should also be applied to these novel chemicals. Using the following combination of keywords in Web of Science and PubMed literature search engines: 'stable isotope' with either 'cathinone', 'legal high', or 'bath salt', two published articles related to stable isotope analysis of synthetic cathinone drug products were generated.

In the first study, quantitative relationship between relative stable isotopic abundances of carbon and hydrogen of the precursor and the product of 4-methylmethcathinone synthesis using the previously mentioned bromination route (see section 1, **Introduction**) was studied [19]. Starting with precursors from two different companies and keeping other chemicals and reaction condition the same, it was discovered that by using this synthetic route, ^{13}C isotopic fractionation factors for the reaction starting from the two precursors were consistent ($\alpha_{net}{}^{13}C_{product/precursor}$ = 0.9966±0.0001 and 0.9967±0.0003)[2] and hence their corresponding

[2] Measurement uncertainties were expressed as ±one standard derivation in the original publication.

isotopic fractionations ($\varepsilon_{net}{}^{13}C_{product/precursor}$ = −3.41±0.11 and −3.35±0.26 mUr)[2,3] (Fig. 6 (a) and (b)). However, there was inconsistency between the ^2H isotopic fractionation factors ($\alpha_{net}{}^{2}H_{product/precursor}$ = 1.0665±0.0026 and 1.0417±0.0011)[2] and hence their corresponding isotopic fractionations ($\varepsilon_{net}{}^{2}H_{product/precursor}$ = 66.5±2.6 and 41.7±1.1 mUr)[2]. This inconsistency was explained by position specific differences in deuterium substitution between the two 1-(4-methylphenyl)propan-1-one precursors at the methylene groups as well as the differences in deuterium compositions of the methylene group hydrogen atoms and those of the rest of the molecule. This study provided the potential to link crime scene synthetic cathinone drug seizures back to the possible precursor materials in clandestine laboratories. However, because the synthesis in the study was carried out under well controlled conditions, this may not represent the reaction conditions being used by clandestine laboratories. Therefore, effects of varying parameters of the reaction condition could be studied in future. Furthermore, this quantitative relationship is established based on the assumption that the final drug products would have to be relatively pure since other chemicals added may alter this relationship as bulk stable isotope analysis is carried out.

In the second publication, forty-nine synthetic cathinone drug seizures (between 2009 and 2014), comprising a hundred and thirty-three samples sourced from Australian Forensic Drug Laboratory of National Measurement Institute Australia, were sampled for analysis of stable isotopes of hydrogen, carbon and nitrogen [55]. These seizures were identified by GC–MS to contain three synthetic cathinones, namely 4-methylmethcathinone, 3,4-methylenedioxymethcathinone and 3,4-methylenedioxypyrovalerone. In a total of nine 3,4-methylenedioxypyrovalerone seizures, there were twenty-two samples analysed for their $\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$ values[4]. From the trivariate plot (Fig. 7), it was observed that ten samples from a single seizure (no. 7) were tightly grouped, suggesting that all samples within a seizure might have originated from the same source. In contrast, it is also possible and logical that samples of two different seizures (e.g. no. 3 and 5 or 8 and 9) could originate from the same source as shown by having the same stable isotopic signature within measurement uncertainties. However, four samples within one seizure (no. 4) were distinguishable, mainly by their very different $\delta^{2}H$ values (−113, −152, −122 and −62 mUr). This situation was explained as different batches of samples were combined for drug trafficking purposes but later seized together by the law enforcement agency. The results suggest that stable isotope analysis allows forensic chemists to provide linkages between different seizures or to exclude any connection between samples. On the other hand, it is important that all $\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$ values should be measured when analysing these drug products because sometimes merely measuring one or two element(s) does not provide enough discriminating power. For examples in the cases of 3,4-methylenedioxymethcathinone seizures, samples 1 ($\delta^{13}C$ = –28.5 mUr, $\delta^{15}N$ = –8.6 mUr, $\delta^{2}H$ = –25 mUr)[5] and 2 ($\delta^{13}C$ = –28.6 mUr, $\delta^{15}N$ = –8.7 mUr, $\delta^{2}H$ = –27 mUr) in seizure 1 were indistinguishable from samples 3 ($\delta^{13}C$ = –28.7 mUr, $\delta^{15}N$ = –11.3 mUr, $\delta^{2}H$ = –27 mUr) and 5 ($\delta^{13}C$ = –28.5 mUr, $\delta^{15}N$ = –10.1 mUr, $\delta^{2}H$ = –28 mUr) in

[3] The unit, mUr, is used throughout this review instead of the traditional notation, ‰, used in the original publications because the use of ‰ has been deprecated [52-54].

[4] The $\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$ values in both publications cited in this review were reported on international scales. The subscripts in $\delta^{13}C_{VPDB}$, $\delta^{15}N_{Air}$, and $\delta^{2}H_{VSMOW}$ are omitted in main text for clarity of reading.

[5] The measurement uncertainties of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$ values were expressed as an expanded uncertainty at 95% confidence interval in the original publication and they are ±0.4, ±0.5 and ±4 mUr respectively. These uncertainties are omitted in main text for clarity of reading.

seizure 4 in terms of $\delta^{13}C$ and $\delta^{2}H$ values but they showed distinguishable $\delta^{15}N$ values; samples 1 to 5 ($\delta^{13}C$ = –29.5 – –29.2 mUr, $\delta^{15}N$ = –5.6 – –5.2 mUr, $\delta^{2}H$ = –15 – –10 mUr) in seizure 3 and samples 1 to 6 in seizure 9 ($\delta^{13}C$ = –30.1 – –29.8 mUr, $\delta^{15}N$ = –5.9 – –5.6 mUr, $\delta^{2}H$ = +9 – +16 mUr) had indistinguishable $\delta^{13}C$ and $\delta^{15}N$ values but the sign of their $\delta^{2}H$ values was reversed. This is the first study to give forensic chemists an insight on relative stable isotopic abundances of synthetic cathinones in drug markets. The $\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$ values of each individual synthetic cathinone drug products in this study are shown in Table 5.

3 Conclusion

Currently there is a limited number of chiral methods for synthetic cathinone analysis with this area continuously evolving. Although the choice of CDs in capillary electrophoretic methods remains controversial, baseline separation of synthetic cathinone enantiomers can be achieved with suitable CDs depending on the experimental condition used. Chiral derivatisations using *L*-TPC and MTPA for GC give baseline separation for a number of synthetic cathinone enantiomers, however these derivatisations were limited to the primary and secondary amine members. Therefore, other chiral derivatisation reagents that can react with tertiary amines should be investigated in order to facilitate chiral separation of the tertiary amine members. On the other hand, research should not be restricted to chiral derivatisation for GC but should also investigate the feasibility of using chiral stationary phases. Native synthetic cathinones can be enantio-separated by HPLC using chiral stationary phases. Polysaccharide phases give baseline separation to many synthetic cathinone enantiomers although some of these phases may not enantio-separate the tertiary amine members. Crown ether phases are found to give high enantio-resolutions, all experimented synthetic cathinone enantiomers were baseline separated with high resolution factors. However, the application was also limited to the primary amine members and hence further investigation on the usefulness of this type of stationary phases on chiral separation of other synthetic cathinones should be carried out. Furthermore, it is also possible to carry out chiral derivatisation for HPLC as an alternative to using chiral stationary phases. Moving from analytical aspect to forensic applications, future research could be focused on areas including quantitative chiral analysis of synthetic cathinones in both physical and biological evidences and isolation of different individual synthetic cathinone enantiomers.

On the area of stable isotope analysis, since there is only limited amount of stable isotope data of synthetic cathinone drug products, more research could be carried out to gain information about the natural variation of relative stable isotopic abundances of these drug products. This can build up the database of literature which can in turn aid linking of drug seizures to their possible sources. Equally important, quantitative stable isotope relationships between these drug products and related chemicals, such as precursor-product relationship, should be investigated to provide complementary information for forensic chemists to distinguish possible sources of the drug products. Detailed studies of this area includes isotope fractionations under different reaction conditions during the synthesis of these drugs. Furthermore, apart from bulk stable isotope analysis, compound specific analysis of these drug products could also be possible. However suitable reference materials are required according to the principle of identical treatment.

Table 1
Summary of publications of chiral analysis of synthetic cathinones by CE

Synthetic cathinone(s)	Detection	Chiral selector(s)	α	R_s	Application(s)	Ref.
Cathinone, Methcathinone	UV	DM-β-CD, SBE-β-CD[a]		1.9 – 3.6	Chiral separation and identification of cathinone, methcathinone, phenalkylamines, cocaine and propoxyphene in forensic exhibits and khat plant materials	[21]
Cathinone, Methcathinone	LIF	NBD-F labelling + HS-γ-CD			Chiral separation of cathinone, methcathinone and phenalkylamines	[24]
Methcathinone	DAD	β-CD			Chiral separation and identification of methcathinone and phenalkylamines in clandestine tablets and urine	[56]
19[b]	DAD	β-CD, γ-CD, S-β-CD[a], CM-β-CD, HP-β-CD	1.019 – 1.229[c]	0.8 – 4.8[c]	Chiral separation of synthetic cathinones	[28]
4-CMC	DAD	S-β-CD, SBE-β-CD			Chiral separation of 4-CMC	[27]
12[b]	MS, DAD	β-CD, HS-γ-CD			Chiral separation and identification of synthetic cathinones in seized drug samples	[23]
19[b]	DAD	β-CD, DM-β-CD, HP-β-CD			Chiral separation and identification of synthetic cathinones and phenethylamines in seized drug samples	[22]
6[b]	MS	HS-γ-CD, (+)-18-C-6-TCA			Chiral separation of secondary amine synthetic cathinones	[25]
4[b]	MS	(+)-18-C-6-TCA		3.6 – 4.0[c,d]	Chiral separation of synthetic cathinones and other controlled substances	[26]
4[b]	DAD	SBE-β-CD	1.015 – 1.049	1.10 – 2.41	Chiral separation of synthetic cathinones and other NPS	[20]
4-MMC, 3,4-MDPV	DAD	α-CD, β-CD, γ-CD, S-β-CD			Chiral separation and quantification of 4-MMC, 4-methylephedrine and 3,4-MDPV in hair	[29]

[a] Separation and/or resolution factor(s) is/are obtained from using this/these chiral selector(s).
[b] Total numbers of synthetic cathinones involved in the studies are shown instead of their identities, please refer to the original articles for their identities.
[c] Not all synthetic cathinones were enantio-separated.
[d] Range is obtained from combining results from two types of CE systems used in the study.

Table 2
Summary of publications of chiral analysis of synthetic cathinones by GC

Synthetic cathinone(s)	Detection	Chiral selector(s)	α	R_s	Application(s)	Ref.
Cathinone	MS	*L*-TPC, (−)-MTPA + DCC, 2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl isothiocyanate, *R*-(+)-α-phenylethyl isocyanate, 2,3,4-triacetyl-α-*D*-arabinopyranosyl isothiocyanate on HP-5MS			Chiral separation and identification of cathinone and phenylalkylamines in over-the-counter cold remedies	[34]
Methcathinone	MS, FID	(+)-MTPA + DCC on DB-5			Chiral separation of methcathinone and phenalkylamines and study of methcathinone racemisation	[6]
Cathinone	MS	*L*-TPC, (+)-MTPA + DCC, (*R*)-(+)-2-chloropropionic acid + DCC, (*S*)-(+)-2-methylbutyric acid + DCC, (*R*)-(−)-2-phenylpropionic acid + DCC, (*R*)-(−)-2-phenylbutyric acid + DCC, (*R*)-(−)-*O*-acetylmandelic acid + DCC, (+)-diacetyl-*L*-tartaric anhydride on DB-5			Chiral separation and identification of cathinone and phenylalkylamines in seized khat plant materials	[33]
Methcathinone	MS	*L*-TPC on HP-1			Confirmation of individual methcathinone enantiomers as the homochiral synthetic products of oxidation of ephedrine and pseudoephedrine	[5]
18[a]	MS	*L*-TPC[b], (*R*)-(−)-MTPA-Cl on HP-5MS	1.005 – 1.067[c]	0.5 – 5.7[c]	Chiral separation of synthetic cathinones	[10]
4-CMC	MS	*L*-TPC on HP-5MS	1.015[d]	0.95[e]	Chiral separation of 4-CMC	[27]
10[a]	MS	*L*-TPC on HP-5MS	1.015 – 1.051[c,f]	0.95 – 2.69[c,f]	Chiral separation of synthetic cathinones and other NPS	[31]
DL-4662	MS	(+)-MTPA + DCC on HP-5MS		19.35	Chiral separation of DL-4662	[35]

[a] Total numbers of synthetic cathinones involved in the studies are shown instead of their identities, please refer to the original articles for their identities.
[b] Separation and/or resolution factor(s) is/are obtained from using this/these chiral selector(s).
[c] Not all chiral separation of synthetic cathinones were carried out under same chromatographic condition.
[d] Separation factor(s) correspond(s) to the best resolution factor(s).
[e] Best resolution factor(s) achieved.
[f] Not all synthetic cathinones were enantio-separated.

31[a]	MS	*L*-TPC on HP-5MS	1.005 – 1.079	1.04 – 14.89	Quantification of synthetic cathinone enantiomers in plasma and urine	[32]
Cathinone	MS	Rt-βDEXsm			Quantification of cathinone, phenylalkylamines and 1-phenylpropan-1,2-dione in khat	[30]

Table 3
Summary of publications of chiral analysis of synthetic cathinones by HPLC

Synthetic cathinone(s)	Detection	Chiral selector(s)	α	R_s	Application(s)	Ref.
Cathinone	DAD	(*S*)-(−)-1-phenylethyl isocyanate on LiChrosorb Si-60			In vivo study of human metabolism of cathinone after oral administration	[36]
Cathinone	DAD	Crownpak CR (+)	7.1	16.96	Chiral separation of cathinone and phenylalkylamines	[39]
Cathinone	DAD	Chiral-CBH-I	1.58[a]	1.08[b]	Chiral separation of cathinone and phenylalkylamines	[57]
12[c]	UV	(+)-18-C-6-TCA with aminopropyl tether, with 3-(*N*-methylamino)propyl tether[d]	1.46 – 2.82	1.50 – 7.17	Chiral separation of primary amine synthetic cathinones	[37]
12[c]	UV	(3,3′-diphenyl-1,1′-binaphthyl)-20-crown-6	1.72 – 8.58	2.60 – 11.10	Chiral separation of primary amine synthetic cathinones	[40]
12[Error! Bookmark not defined.]	UV	*n*-octylethoxysilane + (3,3′-diphenyl-1,1′-binaphthyl)-20-crown-6	2.85 – 16.12	6.49 – 19.64	Chiral separation of primary amine synthetic cathinones	[41]
24[Error! Bookmark not defined.]	DAD	Chiralpak AS-H	1.091 – 3.550[e]	0.5 – 4.5[e]	Chiral separation of synthetic cathinones	[42]
6[Error! Bookmark not defined.]	UV/VIS	Whelk-O 1[d], RegisCell[d], RegisPack[d], Chiralpak AD[d], Chiralcel OD, Chiralcel OJ-H, Chirobiotic V, Cyclobond I 2000 DNP, AmyCoat, CelluCoat	1.22 – 2.46[a,f]	0.83 – 5.90[b,f]	Chiral separation of synthetic cathinones and scale up preparation of 4-MMC enantiomers	[49]
25[Error! Bookmark not defined.]	DAD	2% S-β-CD in mobile phase with LiChrospher RP-18e/8e	1.020 – 1.310[a,e,f]	0.20 – 4.59[b,e,f]	Chiral separation of synthetic cathinones	[58]
DL-4662	DAD	Chiralpak AS-H		2.28	Chiral separation of DL-4662	[35]
14[Error! Bookmark	DAD	Syringic acid, Chiralpak ZWIX(+),	1.03 –	0.7 –	Comparison of different ion	[44]

[a] Separation factor(s) correspond(s) to the best resolution factor(s).
[b] Best resolution factor(s) achieved.
[c] Total numbers of synthetic cathinones involved in the studies are shown instead of their identities, please refer to the original articles for their identities.
[d] Separation and/or resolution factor(s) is/are obtained from using this/these chiral selector(s).
[e] Not all synthetic cathinones were enantio-separated.
[f] Not all chiral separation of synthetic cathinones were carried out under same chromatographic condition.

not defined.		naphthalene based ion exchange type	1.09[a,Error! Bookmark not defined.]	1.86[b,Error! Bookmark not defined.]	exchange type stationary phases for chiral separation of secondary amine synthetic cathinones, β-blockers and anti-malarials	
9[Error! Bookmark not defined.]	UV, Polarimetry	Chiralpak AS-H[d], amylose tris-3,5-dimethylphenylcarbamate[d], amylose tris-3,5-dimethoxyphenylcarbamate, (*S*,*S*)-Whelk-O 1, *L*-Phenylglycine, Chirobiotic T	1.24 – 3.62[Error! Bookmark not defined.]	1.24 – 10.52[Error! Bookmark not defined.]	Determination of chiral ratios of synthetic cathinones in 'legal high' products and scale up preparation of 3,4-MDPV enantiomers	[11]
Cathinone, Methcathinone	MS	Chirobiotic V2			Quantification of cathinone, methcathinone, amphetamine and methamphetamine enantiomers in equine plasma and urine	[45]
Cathinone	DAD	Larihc CF6-P	1.09	1.1	Chiral separation of cathinone, illicit drugs and controlled substances	[46]

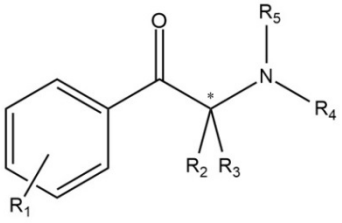
Table 4
Summary of publications of chiral analysis of synthetic cathinones by other analytical methods

Synthetic cathinone(s)	Analytical method(s)	Chiral selector(s)	R_s	Application(s)	Ref.
10[a]	CEC–DAD	Lux Amylose-2[b], Lux Cellulose-4	1.1 – 1.8[c]	Chiral separation of synthetic cathinones	[47]
3[a]	X-Ray Crystallography, HPLC–DAD, Circular Dichroism Spectroscopy, Polarimetry	(+)-*O*,*O*'-di-*p*-toluoyl-*D*-tartaric acid (X-Ray Crystallography), (+)-*O*,*O*'-dibenzoyl-*D*-tartaric acid (X-Ray Crystallography), (−)-*O*,*O*'-dibenzoyl-*L*-tartaric acid (X-Ray Crystallography), Chiralcel OJ-H (HPLC)		Enantioselective isolation of tertiary amine synthetic cathinones	[9]
7[a]	CEC–DAD, HPLC–DAD, SFC–DAD	Chiralcel OD-RH, Chiralcel OZ-H, Chiralpak AD-RH, Lux Amylose-2, Lux Cellulose-1, Lux Cellulose-2, Lux Cellulose-3, Lux Cellulose-4, Sepapak-5	1.73 – 8.19[c,d]	Comparison of different separation techniques for chiral analysis of synthetic cathinones and amphetamine-derivatives	[48]
3,4-MDPV	X-Ray Crystallography, NMR, HPLC–DAD, Polarimetry	(±)-2'-Bromotartranilic acid (X-Ray Crystallography), *R*-(−)-1-phenyl-2,2,2-trifluoroethanol (NMR), Chiralcel OJ (HPLC)		Enantioselective isolation of 3,4-MDPV	[43]

[a] Total numbers of synthetic cathinones involved in the studies are shown instead of their identities, please refer to the original articles for their identities.
[b] Separation and/or resolution factor(s) is/are obtained from using this/these chiral selector(s).
[c] Best resolution factor(s) achieved.
[d] Not all chiral separation of synthetic cathinones were carried out under same chromatographic condition.

Table 5
Ranges of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values of three groups of synthetic cathinone drug products seized between 2009 and 2014 in Australia [55]

	4-MMC	**3,4-MDMC**	**3,4-MDPV**
$\delta^{13}C$ (mUr)	–32.8 – –26.6	–31.2 – –26.1	–31.3 – –28.5
$\delta^{15}N$ (mUr)	–14.1 – –5.2	–11.6 – –2.7	–16.6 – –3.6
$\delta^{2}H$ (mUr)	–68 – +72	–83 – +11	–152 – –35

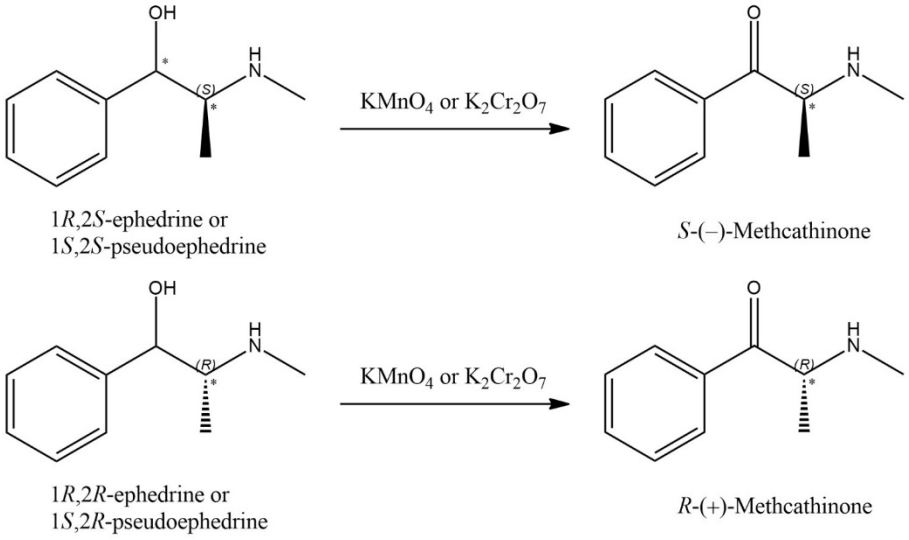


Synthetic cathinone

R1	R2	R3	R4	R5	Common name used in this review	IUPAC nomencalture
H	Me	H	H	H	Cathinone	2-Amino-1-phenylpropan-1-one
H	Me	H	Me	H	Methcathinone	2-(Methylamino)-1-phenylpropan-1-one
H	Me	H	Me	Me	*N,N*-Dimethylcathinone	2-(Dimethylamino)-1-phenylpropan-1-one
H	Me	H	Et	H	Ethcathinone	2-(Ethylamino)-1-phenylpropan-1-one
H	Me	H	Et	Et	*N,N*-Diethylcathinone	2-(Diethylamino)-1-phenylpropan-1-one
H	Et	H	Me	H	Buphedrone	2-(Methylamino)-1-phenylbutan-1-one
H	Pr	H	Me	H	Pentedrone	2-(Methylamino)-1-phenylpentan-1-one
4-Me	Me	H	H	H	4-Methylcathinone	2-Amino-1-(4-methylphenyl)propan-1-one
2-Me	Me	H	Me	H	2-Methylmethcathinone	2-(Methylamino)-1-(2-methylphenyl)propan-1-one
3-Me	Me	H	Me	H	3-Methylmethcathinone	2-(Methylamino)-1-(3-methylphenyl)propan-1-one
4-Me	Me	H	Me	H	4-Methylmethcathinone	2-(Methylamino)-1-(4-methylphenyl)propan-1-one
3,4-Dimethyl	Me	H	Me	H	3,4-Dimethylmethcathinone	1-(3,4-Dimethylphenyl)-2-(methylamino)propan-1-one
2-Me	Me	H	Et	H	2-Methylethcathinone	2-(Ethylamino)-1-(2-methylphenyl)propan-1-one
3-Me	Me	H	Et	H	3-Methylethcathinone	2-(Ethylamino)-1-(3-methylphenyl)propan-1-one
4-Me	Me	H	Et	H	4-Methylethcathinone	2-(Ethylamino)-1-(4-methylphenyl)propan-1-one
2-Et	Me	H	Et	H	2-Ethylethcathinone	2-(Ethylamino)-1-(2-ethylphenyl)propan-1-one
3-Et	Me	H	Et	H	3-Ethylethcathinone	2-(Ethylamino)-1-(3-ethylphenyl)propan-1-one
4-Et	Me	H	Et	H	4-Ethylethcathinone	2-(Ethylamino)-1-(4-ethylphenyl)propan-1-one
3-MeO	Me	H	Me	H	3-Methoxymethcathinone	1-(3-Methoxyphenyl)-2-(methylamino)propan-1-one
4-MeO	Me	H	Me	H	4-Methoxymethcathinone	1-(4-Methoxyphenyl)-2-(methylamino)propan-1-one
3,4-Dimethoxy	Pr	H	Et	H		1-(3,4-Dimethoxyphenyl)-2-(ethylamino)pentan-1-one
4-MeS	Me	H	H	H	4-Methylthiocathinone	2-Amino-1-(4-methylthiophenyl)propan-1-one
4-EtS	Me	H	H	H	4-Ethylthiocathinone	2-Amino-1-(4-ethylthiophenyl)propan-1-one
4-Cl	Me	H	Me	H	4-Chloromethcathinone	1-(4-Chlorophenyl)-2-(methylamino)propan-1-one
3-F	Me	H	Me	H	3-Fluoromethcathinone	1-(3-Fluorophenyl)-2-(methylamino)propan-1-one
4-F	Me	H	Me	H	4-Fluoromethcathinone	1-(4-Fluorophenyl)-2-(methylamino)propan-1-one
3,4-Methylenedioxy	Me	H	Me	H	3,4-Methylenedioxymethcathinone	1-(1,3-Benzodioxol-5-yl)-2-(methylamino)propan-1-one
3,4-Methylenedioxy	Et	H	Me	H	Butylone	1-(1,3-Benzodioxol-5-yl)-2-(methylamino)butan-1-one
3,4-Methylenedioxy	Et	H	Me	Me	*N,N*-Dimethylbutylone	1-(1,3-Benzodioxol-5-yl)-2-(dimethylamino)butan-1-one
H	Me	H	Pyrrolidinyl		α-Pyrrolidinopropiophenone	1-Phenyl-2-(pyrrolidin-1-yl)propan-1-one
4-Me	Me	H	Pyrrolidinyl		4-Methyl-α-pyrrolidinopropiophenone	1-(4-Methylphenyl)-2-(pyrrolidin-1-yl)propan-1-one
4-MeO	Pr	H	Pyrrolidinyl		4-Methoxy-α-pyrrolidinopentiophenone	1-(4-Methoxyphenyl)-2-(pyrrolidin-1-yl)pentan-1-one
3,4-Methylenedioxy	Pr	H	Pyrrolidinyl		3,4-Methylenedioxypyrovalerone	1-(1,3-Benzodioxol-5-yl)-2-(pyrrolidin-1-yl)pentan-1-one
Naphtyl replacing phenyl	Pr	H	Pyrrolidinyl		Naphyrone	1-(Naphthalen-2-yl)-2-(pyrrolidin-1-yl)pentan-1-one
4-F	Pe	H	Pyrrolidinyl		4-Fluoro-α-pyrrolidinoheptiophenone	1-(4-Fluorophenyl)-2-(pyrrolidin-1-yl)heptan-1-one

Fig. 1. General molecular structure of synthetic cathinones. Chiral centre is indicated by asterisk.

(a)



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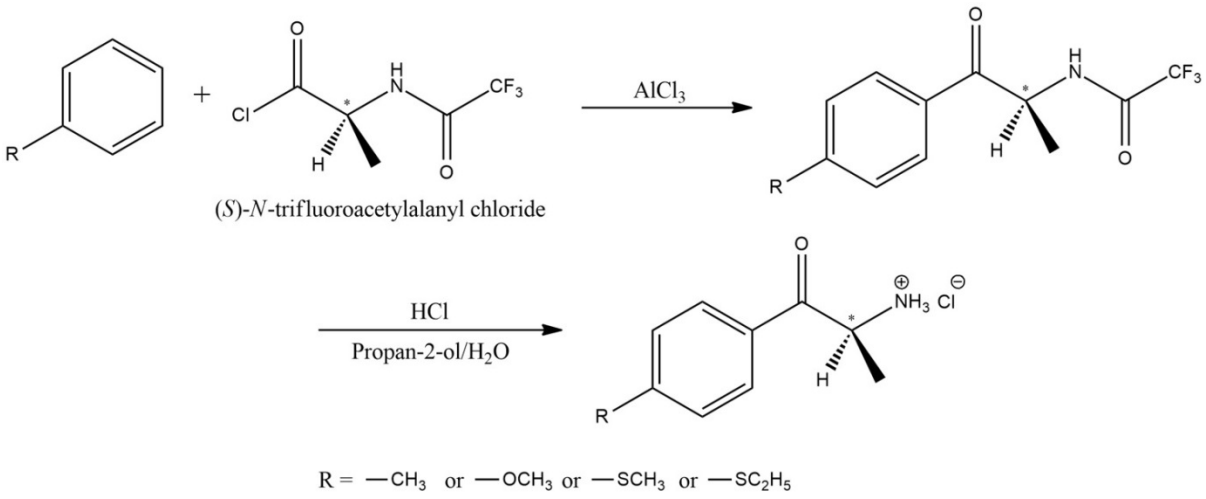


(c)

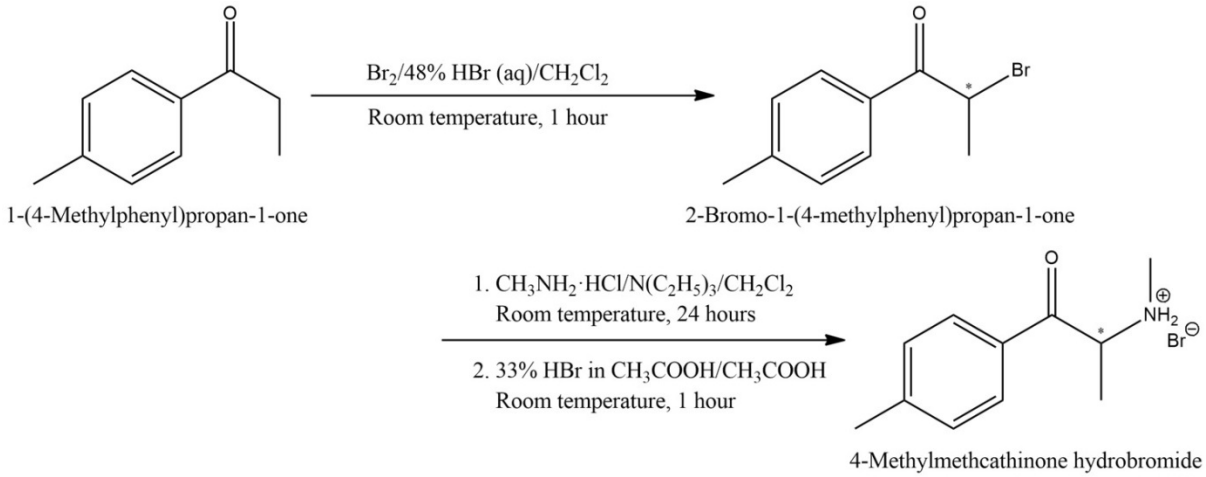


Fig. 2. (a) Homochiral synthesis of methcathinone through oxidation of ephedrines and pseudoephedrines [5]; **(b)** homochiral synthesis of 4-methylcathinone, 4-methoxycathinone, 4-methylthiocathinone and 4-ethylthiocathinone (as hydrochloride salts) through Friedel–

Crafts acylation [7]; **(c)** Achiral synthesis of synthetic cathinones through bromination followed by nucleophilic substitution (exemplified as 4-methylmethcathinone hydrobromide synthesis) [19]. Chiral centres are indicated by asterisk.

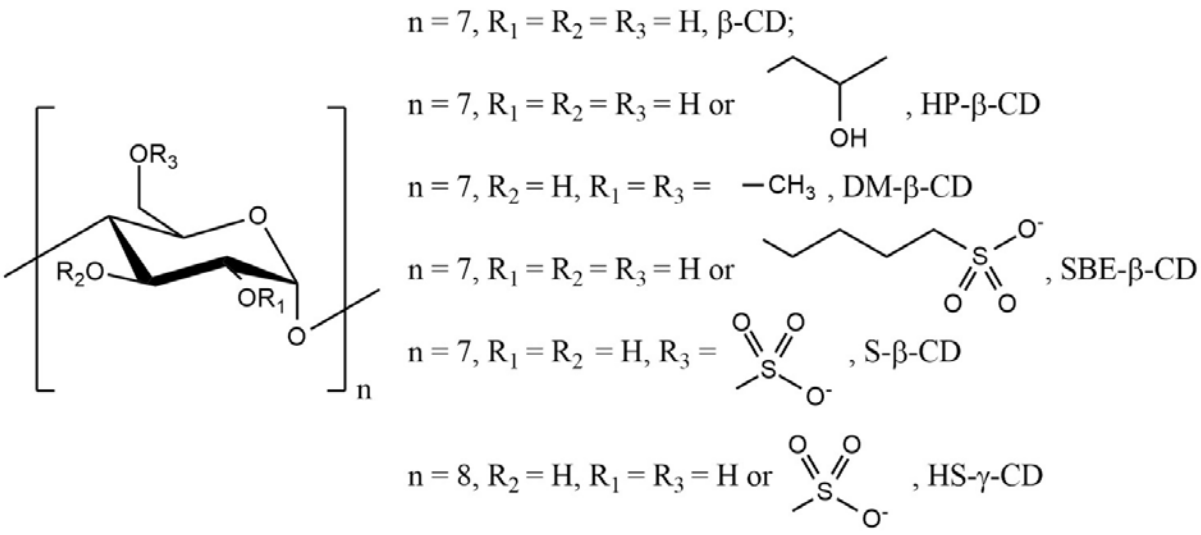


Fig. 3. CDs used in CE for chiral separation of synthetic cathinones. Note that for sulfated- and highly sulfated-CDs, the actual numbers and positions of sulfate groups attached to CD backbones could vary.

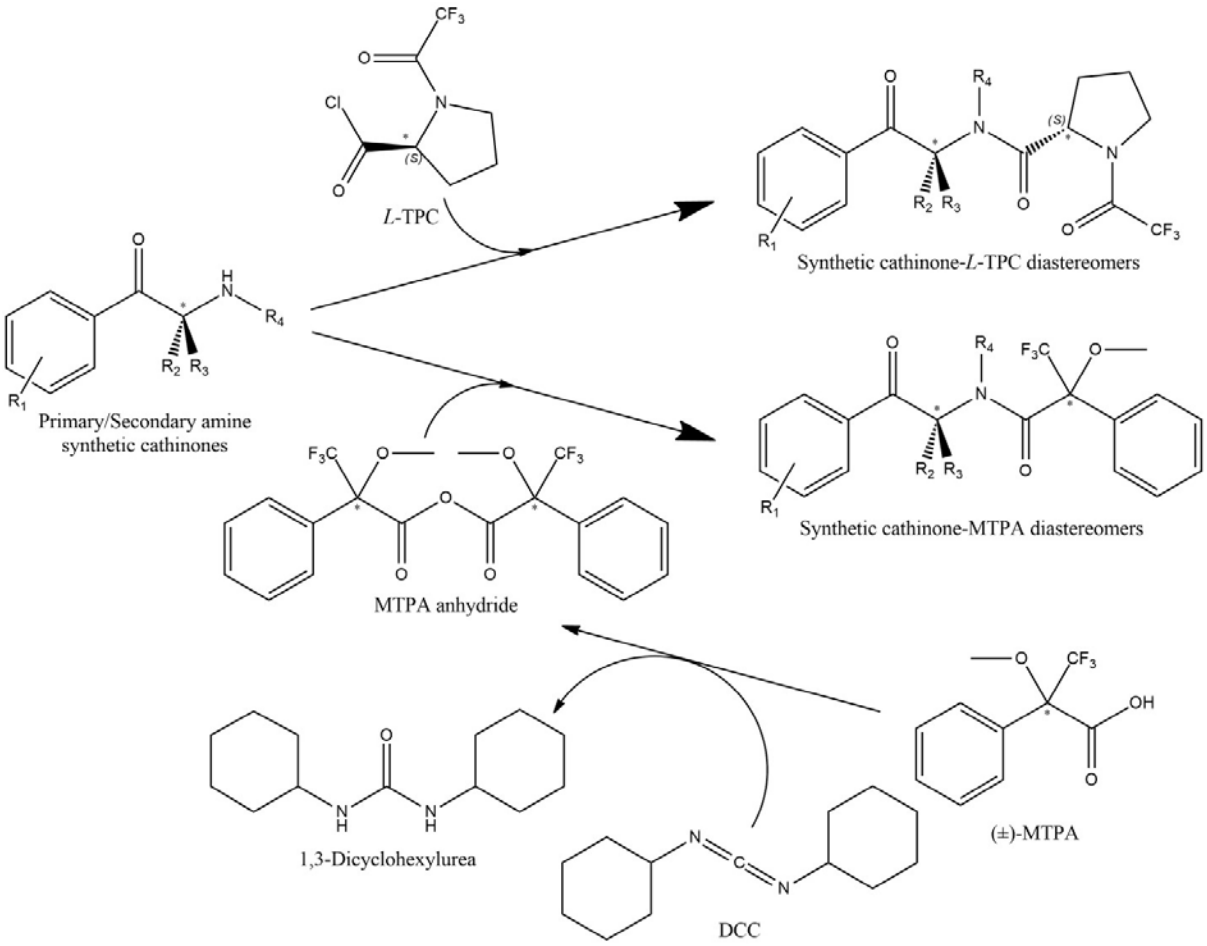
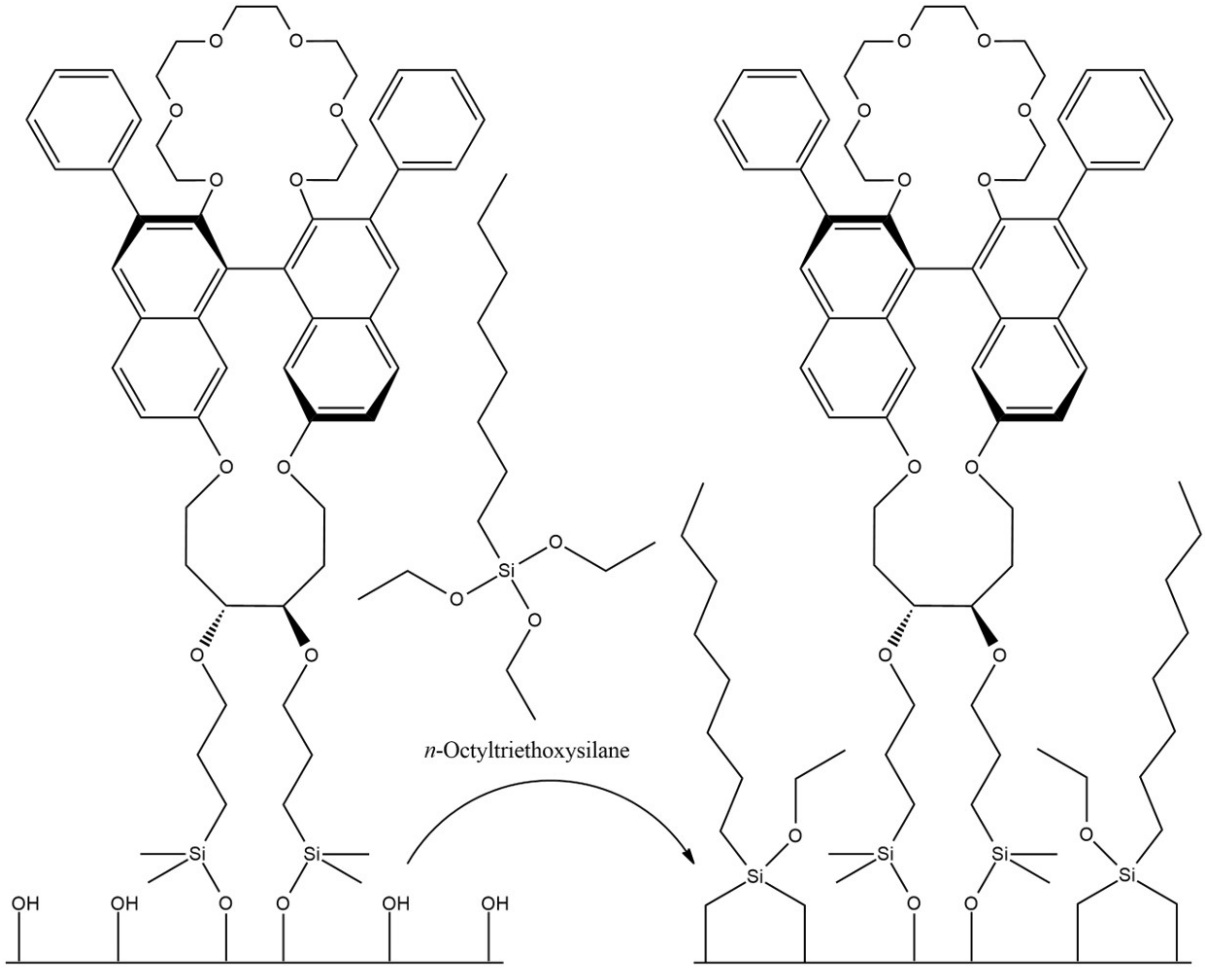


Fig. 4. Chiral derivatisation of primary or secondary amine synthetic cathinones by *L*-TPC and MTPA anhydride, where the latter one is prepared in situ by reacting the original MTPA with DCC. Chiral centres are indicated by asterisk.

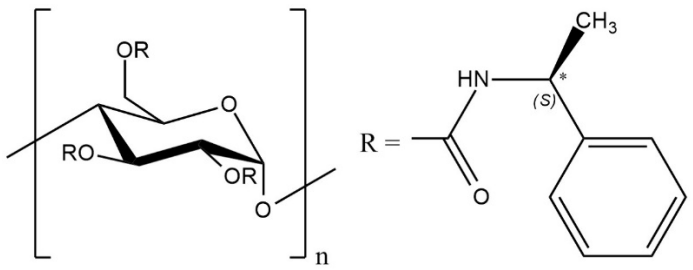
(a)



(3,3'-Diphenyl-1,1'-binaphthyl)-20-crown-6 based stationary phase, with residual silanol groups on silica gel

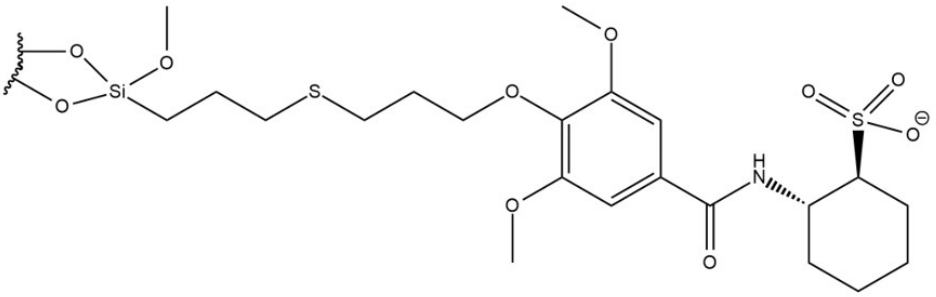
(3,3'-Diphenyl-1,1'-binaphthyl)-20-crown-6 based stationary phase, with the residual silanol groups on silica gel protected by *n*-octyl groups

(b)

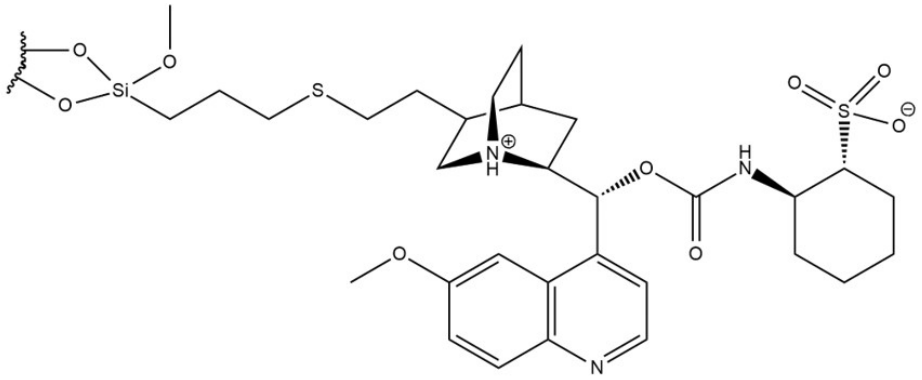


Amylose tris [(*S*)-α-methylbenzylcarbamate] stationary phase

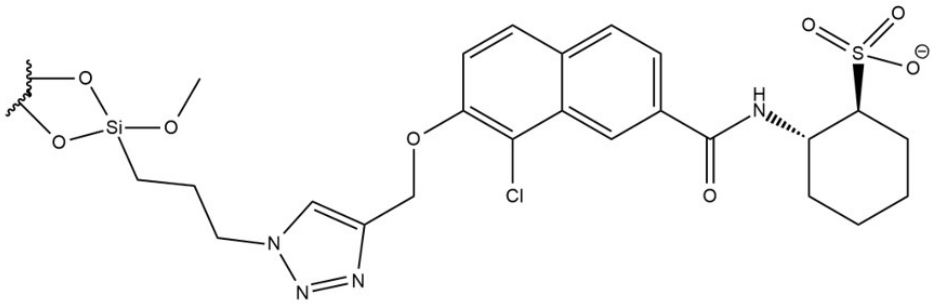
(c)



Syringic acid based ion exchange stationary phase



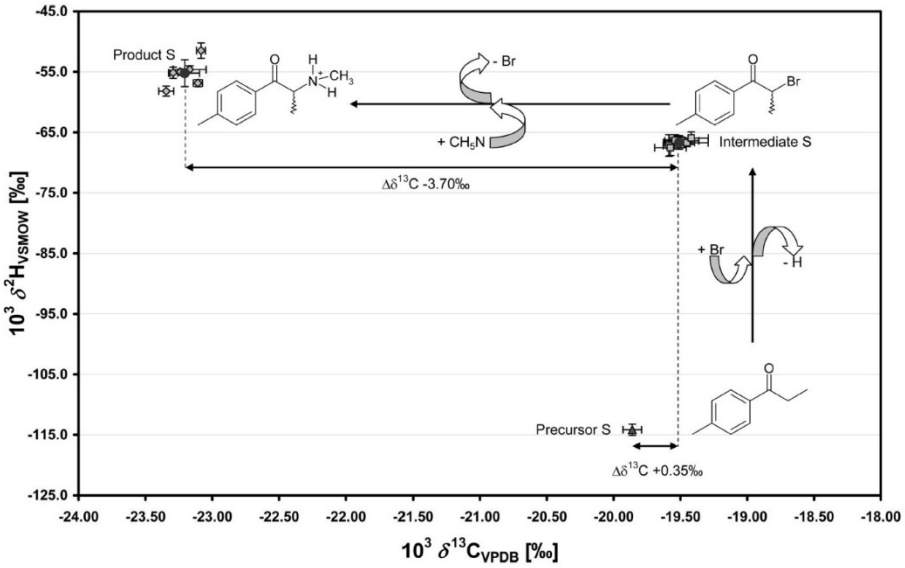
Chiralpak ZWIX(+) based ion exchange stationary phase



Naphthalene based ion exchange stationary phase

Fig. 5. Chiral stationary phases used in HPLC for chiral separation of synthetic cathinones. **(a)** crown ether phase, (3,3'-diphenyl-1,1'-binaphthyl)-20-crown-6, with and without *n*-octyl groups protecting the residual silanol groups of silica gel [41]; **(b)** polysaccharide phase, amylose tris [(*S*)-α-methylbenzylcarbamate], on commercial Chiralpak AS-H column (chiral centre is indicated by asterisk); **(c)** three ion exchange phases, which are syringic acid, Chiralpak ZWIX(+), and naphthalene based [44].

(a)



(b)

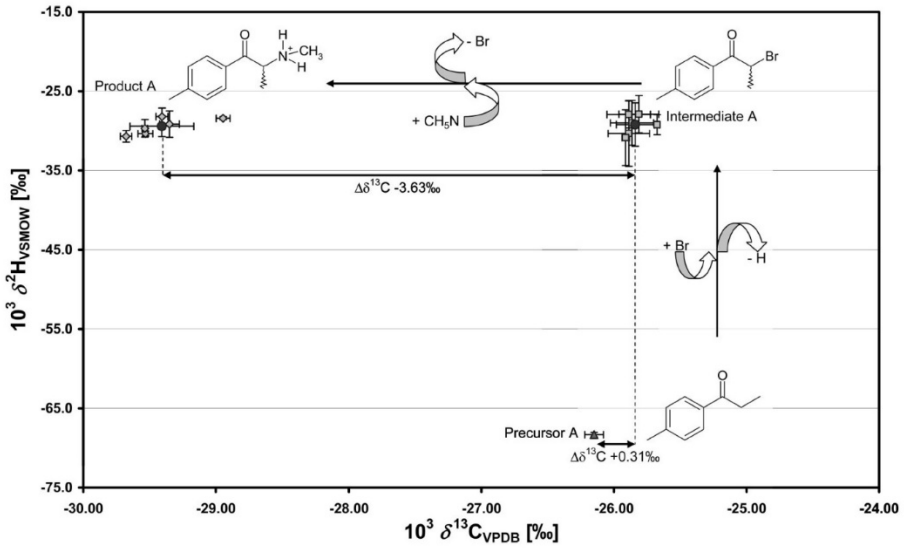


Fig. 6. Consistent quantitative relationship between $\delta^{13}C$ values of the precursor and the product of 4-methylmethcathinone synthesis through the bromination route. Change of $\delta^{13}C$ value throughout the synthesis starting from the precursor purchased from (a) company S and (b) company A. Reprinted with permission from NicDaéid, N. et al. *Anal. Chem.* **2012**, 84, 8691-8696. Copyright (2012) American Chemical Society.

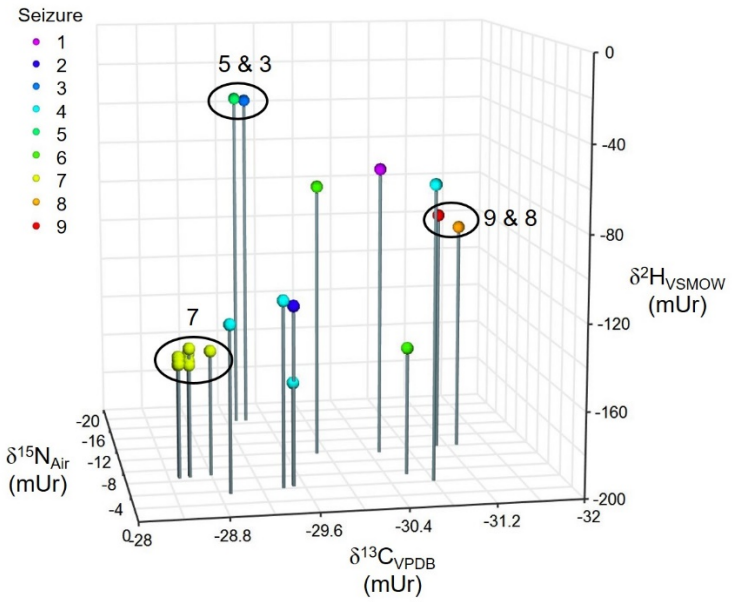


Fig. 7. Trivariate plot of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values of 3,4-MDPV product seized between 2009 and 2014 in Australia. Samples within one ellipse could be grouped because of their indistinguishable or very similar stable isotopic signatures. Replotted from the tabulated data obtained from the original article [55]. Colour is required for this figure.

References

Uncategorized References

[1] European Monitoring Centre for Drugs and Drug Addiction, European Drug Report 2016: Trends and Developments, (2016). DOI http://dx.doi.org/10.2810/04312
[2] D. Zuba, Identification of cathinones and other active components of 'legal highs' by mass spectrometric methods, Trends in Analytical Chemistry, 32 (2012) 15-30. DOI http://dx.doi.org/10.1016/j.trac.2011.09.009
[3] J.P. Smith, O.B. Sutcliffe, C.E. Banks, An overview of recent developments in the analytical detection of new psychoactive substances (NPSs), Analyst, 140 (2015) 4932-4948. DOI http://dx.doi.org/10.1039/c5an00797f
[4] A. Namera, M. Kawamura, A. Nakamoto, T. Saito, M. Nagao, Comprehensive review of the detection methods for synthetic cannabinoids and cathinones, Forensic Toxicology, 33 (2015) 175-194. DOI http://dx.doi.org/10.1007/s11419-015-0270-0
[5] J. DeRuiter, L. Hayes, A. Valaer, C.R. Clark, F.T. Noggle, Methcathinone and designer analogues: Synthesis, stereochemical analysis, and analytical properties, J Chromatogr Sci, 32 (1994) 552-564. DOI http://dx.doi.org/10.1093/chromsci/32.12.552
[6] M.J. LeBelle, C. Savard, B.A. Dawson, D.B. Black, L.K. Katyal, F. Zrcek, A.W. By, Chiral identification and determination of ephedrine, pseudoephedrine, methamphetamine and metecathinone by gas chromatography and nuclear magnetic resonance, Forensic Science International, 71 (1995) 215-223. DOI http://dx.doi.org/10.1016/0379-0738(94)01669-0
[7] M. Osorio-Olivares, M.C. Rezende, S. Sepúlveda-Boza, B.K. Cassels, R.F. Baggio, J.C. Muñoz-Acevedo, A two-step method for the preparation of homochiral cathinones, Tetrahedron: Asymmetry, 14 (2003) 1473-1477. DOI http://dx.doi.org/10.1016/S0957-4166(03)00317-3
[8] M. Reist, L.H. Christiansen, P. Christoffersen, P.-A. Carrupt, B. Testa, Low configurational stability of amfepramone and cathinone: Mechanism and kinetics of chiral inversion, Chirality, 7 (1995) 469-473. DOI http://dx.doi.org/10.1002/chir.530070613
[9] M.J. Zhou, S. Bouazzaoui, L.E. Jones, P. Goodrich, S.E. Bell, G.N. Sheldrake, P.N. Horton, S.J. Coles, N.C. Fletcher, Isolation and structural determination of non-racemic tertiary cathinone derivatives, Org Biomol Chem, 13 (2015) 9629-9636. DOI http://dx.doi.org/10.1039/c5ob01306b
[10] S. Mohr, J.A. Weiß, J. Spreitz, M.G. Schmid, Chiral separation of new cathinone- and amphetamine-related designer drugs by gas chromatography–mass spectrometry using trifluoroacetyl-*L*-prolyl chloride as chiral derivatization reagent, Journal of Chromatography A, 1269 (2012) 352-359. DOI http://dx.doi.org/10.1016/j.chroma.2012.09.079
[11] B. Silva, C. Fernandes, M.E. Tiritan, M.M.M. Pinto, M.J. Valente, M. Carvalho, P.G. de Pinho, F. Remião, Chiral enantioresolution of cathinone derivatives present in "legal highs", and enantioselectivity evaluation on cytotoxicity of 3,4-methylenedioxypyrovalerone (MDPV), Forensic Toxicology, 34 (2016) 372-385. DOI http://dx.doi.org/10.1007/s11419-016-0324-y
[12] P. Kalix, The releasing effect of the isomers of the alkaloid cathinone at central and peripheral catecholamine storage sites, Neuropharmacology, 25 (1986) 499-501. DOI http://dx.doi.org/10.1016/0028-3908(86)90174-7
[13] R.A. Glennon, R. Young, B.R. Martin, T.A. Dal Cason, Methcathione ("cat"): An enantiomeric potency comparison, Pharmacology Biochemistry and Behavior, 50 (1995) 601-606. DOI http://dx.doi.org/10.1016/0091-3057(94)00348-3
[14] R.A. Gregg, M.H. Baumann, J.S. Partilla, J.S. Bonano, A. Vouga, C.S. Tallarida, V. Velvadapu, G.R. Smith, M.M. Peet, A.B. Reitz, S.S. Negus, S.M. Rawls, Stereochemistry of mephedrone neuropharmacology: Enantiomer-specific behavioral and neurochemical effects in rats, Br J Pharmacol, 172 (2015) 883-894. DOI http://dx.doi.org/10.1111/bph.12951
[15] J.P. Smith, J.P. Metters, C. Irving, O.B. Sutcliffe, C.E. Banks, Forensic electrochemistry: The electroanalytical sensing of synthetic cathinone-derivatives and their accompanying adulterants in "legal high" products, Analyst, 139 (2014) 389-400. DOI http://dx.doi.org/10.1039/c3an01985c
[16] D. Hamby, A. Burnett, M. Jablonsky, B. Twamley, P.V. Kavanagh, E.A. Gardner, Identification of 2-(ethylamino)-1-(4-methylphenyl)-1-pentanone (4-MEAP), a new "legal high" sold by an Internet

vendor as 4-methyl pentedrone, Journal of Forensic Sciences, 60 (2015) 721-726. DOI http://dx.doi.org/10.1111/1556-4029.12712
[17] S.D. McDermott, J.D. Power, P. Kavanagh, J. O'Brien, The analysis of substituted cathinones. Part 2: An investigation into the phenylacetone based isomers of 4-methylmethcathinone and *N*-ethylcathinone, Forensic Science International, 212 (2011) 13-21. DOI http://dx.doi.org/10.1016/j.forsciint.2011.06.030
[18] S. Davis, K. Rands-Trevor, S. Boyd, M. Edirisinghe, The characterisation of two halogenated cathinone analogues: 3,5-Difluoromethcathinone and 3,5-dichloromethcathinone, Forensic Science International, 217 (2012) 139-145. DOI http://dx.doi.org/10.1016/j.forsciint.2011.10.042
[19] N. NicDaéid, W. Meier-Augenstein, H.F. Kemp, O.B. Sutcliffe, Using isotopic fractionation to link precursor to product in the synthesis of (±)-mephedrone: A new tool for combating "legal high" drugs, Analytical Chemistry, 84 (2012) 8691-8696. DOI http://dx.doi.org/10.1021/ac3019069
[20] M. Taschwer, M.G. Hofer, M.G. Schmid, Enantioseparation of benzofurys and other novel psychoactive compounds by CE and sulfobutylether beta-cyclodextrin as chiral selector added to the BGE, ELECTROPHORESIS, 35 (2014) 2793-2799. DOI http://dx.doi.org/10.1002/elps.201400164
[21] I.S. Lurie, R.F. Klein, T.A. Dal Cason, M.J. LeBelle, R. Brenneisen, R.E. Weinberger, Chiral resolution of cationic drugs of forensic interest by capillary electrophoresis with mixtures of neutral and anionic cyclodextrins, Analytical Chemistry, 66 (1994) 4019-4026. DOI http://dx.doi.org/10.1021/ac00094a026
[22] L. Li, I.S. Lurie, Regioisomeric and enantiomeric analyses of 24 designer cathinones and phenethylamines using ultra high performance liquid chromatography and capillary electrophoresis with added cyclodextrins, Forensic Science International, 254 (2015) 148-157. DOI http://dx.doi.org/10.1016/j.forsciint.2015.06.026
[23] G. Merola, H. Fu, F. Tagliaro, T. Macchia, B.R. McCord, Chiral separation of 12 cathinone analogs by cyclodextrin-assisted capillary electrophoresis with UV and mass spectrometry detection, ELECTROPHORESIS, 35 (2014) 3231-3241. DOI http://dx.doi.org/10.1002/elps.201400077
[24] S.R. Wallenborg, I.S. Lurie, D.W. Arnold, C.G. Bailey, On-chip chiral and achiral separation of amphetamine and related compounds labeled with 4-fluoro-7-nitrobenzofurazane, ELECTROPHORESIS, 21 (2000) 3257-3263. DOI http://dx.doi.org/10.1002/1522-2683(20000901)21:15<3257::aid-elps3257>3.0.co;2-m
[25] M. Moini, C.M. Rollman, Compatibility of highly sulfated cyclodextrin with electrospray ionization at low nanoliter/minute flow rates and its application to capillary electrophoresis/electrospray ionization mass spectrometric analysis of cathinone derivatives and their optical isomers, Rapid Communications in Mass Spectrometry, 29 (2015) 304-310. DOI http://dx.doi.org/10.1002/rcm.7106
[26] C.M. Rollman, M. Moini, Ultrafast capillary electrophoresis/mass spectrometry of controlled substances with optical isomer separation in about a minute, Rapid Communications in Mass Spectrometry, 30 (2016) 2070-2076. DOI http://dx.doi.org/10.1002/rcm.7691
[27] M. Taschwer, J.A. Weiß, O. Kunert, M.G. Schmid, Analysis and characterization of the novel psychoactive drug 4-chloromethcathinone (clephedrone), Forensic Science International, 244 (2014) e56-59. DOI http://dx.doi.org/10.1016/j.forsciint.2014.09.007
[28] S. Mohr, S. Pilaj, M.G. Schmid, Chiral separation of cathinone derivatives used as recreational drugs by cyclodextrin-modified capillary electrophoresis, ELECTROPHORESIS, 33 (2012) 1624-1630. DOI http://dx.doi.org/10.1002/elps.201100570
[29] T. Baciu, F. Borrull, M. Calull, C. Aguilar, Enantioselective determination of cathinone derivatives in human hair by capillary electrophoresis combined in-line with solid-phase extraction, ELECTROPHORESIS, (2016). DOI http://dx.doi.org/10.1002/elps.201600149
[30] R. Krizevski, N. Dudai, E. Bar, I. Dessow, U. Ravid, E. Lewinsohn, Quantitative stereoisomeric determination of phenylpropylamino alkaloids in khat (Catha edulis Forsk.) using chiral GC–MS, Israel Journal of Plant Sciences, 56 (2008) 207-213. DOI http://dx.doi.org/10.1560/IJPS.56.3.207

[31] J.A. Weiß, S. Mohr, M.G. Schmid, Indirect chiral separation of new recreational drugs by gas chromatography–mass spectrometry using trifluoroacetyl-*L*-prolyl chloride as chiral derivatization reagent, Chirality, 27 (2015) 211-215. DOI http://dx.doi.org/10.1002/chir.22414
[32] R.H. Alremeithi, M.A. Meetani, S.A. Khalil, A validated gas chromatography mass spectrometry method for simultaneous determination of cathinone related drug enantiomers in urine and plasma, RSC Advances, 6 (2016) 80576-80584. DOI http://dx.doi.org/10.1039/C6RA10583A
[33] M.J. LeBelle, G. Lauriault, A. Lavoie, Gas chromatographic–mass spectrometric identification of chiral derivatives of the alkaloids of KHAT, Forensic Science International, 61 (1993) 53-64. DOI http://dx.doi.org/10.1016/0379-0738(93)90249-A
[34] S.-M. Wang, R.J. Lewis, D. Canfield, T.-L. Li, C.-Y. Chen, R.H. Liu, Enantiomeric determination of ephedrines and norephedrines by chiral derivatization gas chromatography–mass spectrometry approaches, Journal of Chromatography B, 825 (2005) 88-95. DOI http://dx.doi.org/10.1016/j.jchromb.2005.01.016
[35] J.A. Weiß, M. Taschwer, O. Kunert, M.G. Schmid, Analysis of a new drug of abuse: Cathinone derivative 1-(3,4-dimethoxyphenyl)-2-(ethylamino)pentan-1-one, Journal of Separation Science, 38 (2015) 825-828. DOI http://dx.doi.org/10.1002/jssc.201401052
[36] K. Mathys, R. Brenneisen, Determination of (*S*)-(−)-cathinone and its metabolites (*R*,*S*)-(−)-norephedrine and (*R*,*R*)-(−)-norpseudoephedrine in urine by high-performance liquid chromatography with photodiode-array detection, Journal of Chromatography A, 593 (1992) 79-85. DOI http://dx.doi.org/10.1016/0021-9673(92)80270-5
[37] M.H. Hyun, G. Tan, Y.J. Cho, Liquid chromatographic resolution of aryl α-amino ketones on chiral stationary phases based on (+)-(18-Crown-6)-2,3,11,12-tetracarboxylic acid, Journal of Liquid Chromatography & Related Technologies, 27 (2004) 1671-1680. DOI http://dx.doi.org/10.1081/JLC-120037363
[38] M.H. Hyun, Y. Jae Cho, J. Ah Kim, J. Sung Jin, Preparation and application of a new modified liquid chromatographic chiral stationary phase based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid, Journal of Chromatography A, 984 (2003) 163-171. DOI http://dx.doi.org/10.1016/S0021-9673(02)01833-2
[39] H.Y. Aboul-Enein, V. Serignese, Direct chiral resolution of phenylalkylamines using a crown ether chiral stationary phase, Biomedical Chromatography, 11 (1997) 7-10. DOI http://dx.doi.org/10.1002/(sici)1099-0801(199701)11:1<7::aid-bmc607>3.0.co;2-f
[40] M. Ho Hyun, G. Tan, Y.J. Cho, Liquid chromatographic enantioseparation of aryl α-amino ketones on a crown ether-based chiral stationary phase, Biomedical Chromatography, 19 (2005) 208-213. DOI http://dx.doi.org/10.1002/bmc.437
[41] H.J. Choi, J.S. Jin, M.H. Hyun, Liquid chromatographic direct resolution of aryl α-amino ketones on a residual silanol group-protecting chiral stationary phase based on optically active (3,3'-diphenyl-1,1'-binaphthyl)-20-crown-6, Journal of Chromatography B, 875 (2008) 102-107. DOI http://dx.doi.org/10.1016/j.jchromb.2008.05.047
[42] S. Mohr, M. Taschwer, M.G. Schmid, Chiral separation of cathinone derivatives used as recreational drugs by HPLC–UV using a CHIRALPAK® AS-H column as stationary phase, Chirality, 24 (2012) 486-492. DOI http://dx.doi.org/10.1002/chir.22048
[43] M. Suzuki, J.R. Deschamps, A.E. Jacobson, K.C. Rice, Chiral resolution and absolute configuration of the enantiomers of the psychoactive "designer drug" 3,4-methylenedioxypyrovalerone, Chirality, 27 (2015) 287-293. DOI http://dx.doi.org/10.1002/chir.22423
[44] D. Wolrab, P. Frühauf, A. Moulisová, M. Kuchař, C. Gerner, W. Lindner, M. Kohout, Chiral separation of new designer drugs (cathinones) on chiral ion-exchange type stationary phases, J Pharm Biomed Anal, 120 (2016) 306-315. DOI http://dx.doi.org/10.1016/j.jpba.2015.12.023
[45] C. Wang, P. Hartmann-Fischbach, T. Krueger, A. Lester, A. Simonson, T. Wells, M. Wolk, N. Hidlay, Fast and sensitive chiral analysis of amphetamines and cathinones in equine urine and plasma using liquid chromatography tandem mass spectrometry, American Journal of Analytical Chemistry, 6 (2015) 995-1003. DOI http://dx.doi.org/10.4236/ajac.2015.613094

[46] N.L. Padivitage, E. Dodbiba, Z.S. Breitbach, D.W. Armstrong, Enantiomeric separations of illicit drugs and controlled substances using cyclofructan-based (LARIHC) and cyclobond I 2000 RSP HPLC chiral stationary phases, Drug Test Anal, 6 (2014) 542-551. DOI http://dx.doi.org/10.1002/dta.1534
[47] Z. Aturki, M.G. Schmid, B. Chankvetadze, S. Fanali, Enantiomeric separation of new cathinone derivatives designer drugs by capillary electrochromatography using a chiral stationary phase, based on amylose tris(5-chloro-2-methylphenylcarbamate), ELECTROPHORESIS, 35 (2014) 3242-3249. DOI http://dx.doi.org/10.1002/elps.201400085
[48] D. Albals, Y.V. Heyden, M.G. Schmid, B. Chankvetadze, D. Mangelings, Chiral separations of cathinone and amphetamine-derivatives: Comparative study between capillary electrochromatography, supercritical fluid chromatography and three liquid chromatographic modes, J Pharm Biomed Anal, 121 (2016) 232-243. DOI http://dx.doi.org/10.1016/j.jpba.2015.12.007
[49] R.W. Perera, I. Abraham, S. Gupta, P. Kowalska, D. Lightsey, C. Marathaki, N.S. Singh, W.J. Lough, Screening approach, optimisation and scale-up for chiral liquid chromatography of cathinones, Journal of Chromatography A, 1269 (2012) 189-197. DOI http://dx.doi.org/10.1016/j.chroma.2012.11.001
[50] J.F. Carter, W. Meier-Augenstein, Stable isotope analysis: Drugs, Wiley Encyclopedia of Forensic Science, John Wiley & Sons, Ltd, 2009. DOI http://dx.doi.org/10.1002/9780470061589.fsa1065
[51] W. Meier-Augenstein, Distinguishing drugs, Stable Isotope Forensics: An Introduction to the Forensic Application of Stable Isotope Analysis, John Wiley & Sons, Ltd, 2010, pp. 149-168. DOI http://dx.doi.org/10.1002/9780470688762.ch14
[52] E.R. Cohen, T. Cvitaš, J.G. Frey, B. Holmström, K. Kuchitsu, R. Marquardt, I. Mills, F. Pavese, M. Quack, J. Stohner, H.L. Strauss, M. Takami, A.J. Thor, Definitions and symbols for units, Quantities, Units and Symbols in Physical Chemistry, IUPAC Green Book, 3rd Edition, 2nd Printing, IUPAC and RSC Publishing, Cambridge, United Kingdom, 2007, pp. 84-100. DOI http://dx.doi.org/10.1039/9781847557889-00181
[53] W.A. Brand, T.B. Coplen, Stable isotope deltas: Tiny, yet robust signatures in nature, Isotopes in Environmental and Health Studies, 48 (2012) 393-409. DOI http://dx.doi.org/10.1080/10256016.2012.666977
[54] A. Schimmelmann, H. Qi, T.B. Coplen, W.A. Brand, J. Fong, W. Meier-Augenstein, H.F. Kemp, B. Toman, A. Ackermann, S. Assonov, A.T. Aerts-Bijma, R. Brejcha, Y. Chikaraishi, T. Darwish, M. Elsner, M. Gehre, H. Geilmann, M. Gröning, J.-F. Hélie, S. Herrero-Martín, H.A.J. Meijer, P.E. Sauer, A.L. Sessions, R.A. Werner, Organic reference materials for hydrogen, carbon, and nitrogen stable isotope-ratio measurements: Caffeines, n-alkanes, fatty acid methyl esters, glycines, *L*-Valines, polyethylenes, and oils, Analytical Chemistry, 88 (2016) 4294-4302. DOI http://dx.doi.org/10.1021/acs.analchem.5b04392
[55] M. Collins, A. Doddridge, H. Salouros, Cathinones: Isotopic profiling as an aid to linking seizures, Drug Test Anal, (2015). DOI http://dx.doi.org/10.1002/dta.1886
[56] A.-S. Liau, J.-T. Liu, L.-C. Lin, Y.-C. Chiu, Y.-R. Shu, C.-C. Tsai, C.-H. Lin, Optimization of a simple method for the chiral separation of methamphetamine and related compounds in clandestine tablets and urine samples by β-cyclodextrine modified capillary electrophoresis: A complementary method to GC–MS, Forensic Science International, 134 (2003) 17-24. DOI http://dx.doi.org/10.1016/S0379-0738(03)00096-3
[57] H.Y. Aboul-Enein, V. Serignese, Direct enantiomeric separation of cathinone and one major metabolite on cellobiohydrolase (CBH-I) chiral stationary phase, Biomedical Chromatography, 11 (1997) 47-49. DOI http://dx.doi.org/10.1002/(sici)1099-0801(199701)11:1<47::aid-bmc626>3.0.co;2-m
[58] M. Taschwer, Y. Seidl, S. Mohr, M.G. Schmid, Chiral separation of cathinone and amphetamine derivatives by HPLC/UV using sulfated β-cyclodextrin as chiral mobile phase additive, Chirality, 26 (2014) 411-418. DOI http://dx.doi.org/10.1002/chir.22341