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# Differentiation of the six dimethoxypyrovalerone regioisomers: GC-MS, GC-MS/MS and GC-IR

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ABSTRACT

Multiple and complementary analytical methods are often necessary for the identification of a specific compound from a series of closely related structural isomers. Gas chromatography-mass spectrometry (GC-MS), gas chromatography-product ion mass spectrometry (GC-MS/MS) and gas chromatography-infrared spectroscopy (GC-IR) were used to differentiate between the six dimethoxypyrrovalerone (DMPV) regioisomers. The six regioisomeric aminoketones were separated on a 50% phenyl stationary phase and the elution order is related to the positioning of substituents on the aromatic ring. These six DMPV regioisomers yield essentially identical mass spectral data in both chemical ionization (CI-MS) and electron ionization (EI-MS) spectra as well as identical product ion MS/MS spectra of the iminium cation base peak (*m/z* 126). These various mass spectral techniques provide data to identify all major structural features of these molecules except the dimethoxy substitution pattern of the aromatic ring. The region of the vapor phase infrared spectra between 1600 $cm^{-1}$ and 1000 $cm^{-1}$ provides a significant number of unique absorption bands characteristic of each individual DMPV regioisomer.

## 1. Introduction

The United Nations Office on Drugs and Crime reported a total of 75 new psychoactive substances appearing for the first time in 2015 according to the 2016 World Drug Report [1]. This represents an increase from the 66 new psychoactive substances from clandestine samples reported during the previous year. A total of 20 of these first time reports in 2015 represented new synthetic cathinone derivatives and for the first time in history the number of new synthetic cathinones rivaled the number of reported new synthetic cannabinoids, 20 and 21 respectively. In previous years (2012–2014) the vast majority of first time reports were for synthetic cannabinoids.

The human behavioral effects of the cathinone derivatives are primarily related to central nervous system stimulation [2] and can include agitation, anxiety, confusion, euphoria and hallucinations. The relative intensity of these effects varies with the individual compound and dose [2,3]. The synthetic cathinones produce CNS stimulation by increasing synaptic catecholamine concentrations [4] by a variety of processes including neurotransmitter release as well as inhibition of monoamine re-uptake transporters for dopamine, norepinephrine, and serotonin [2–8]. Many of the potent pyrrolidine containing cathinone derivatives (the pyrovalerone compounds) are selective inhibitors of neurotransmitter re-uptake transporter proteins [4,9]. This unique selectivity appears to be due in part to the presence of the pyrrolidine ring in these designer cathinone derivatives.

The designer style molecular modification and synthesis of analogues in a number of drug categories are possible due to the commercial availability of many regioisomeric forms of common precursor substances. A single synthetic pathway utilizing a variety of regioisomeric precursor materials can yield numerous closely related products. These compounds in many cases have identical molecular framework, elemental composition, functional groups and often equivalent or identical mass spectral fragments. Chromatographic co-elution of regioisomeric substances that yield regioisomeric or identical mass spectral fragment ions would require a unique strategy for identification.

The use of multiple and complementary analytical methods such as GC-MS and GC-IR are often necessary for the specific identification of one compound from a series of closely related structural isomers. Aromatic ring substitution patterns often yield unique molecular fingerprints in their infrared spectra. Vapor phase GC-IR techniques generally provide differentiation of aromatic ring substitution patterns following MS data to focus on a specific set of regioisomeric substances.

One of the major directions of molecular manipulation in designer

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drug evolution has been the addition of small alkyl ethers to unsubstituted aromatic rings in several drug categories. A common example of this type of modification is the insertion of the methylenedioxy moiety to amphetamine, methamphetamine and alpha-pyrrolidinovalerophenone (alpha-PVP) producing MDA, MDMA and 3,4-methylenedioxypyrovalerone (MDPV), respectively [3,8,10–13]. The addition of a dimethoxy group to the basic phenethylamine structure followed by aromatic ring halogenation has led to the series of substances of abuse known as 2C-B or 2C-I [8,14]. Further modification of this series by addition of a methoxybenzyl group to the amine functionality of the 2-C series has produced another group of hallucinogenic drugs with an LSD-like activity spectrum referred to as the N-BOMe drugs [15–19].

The rapid proliferation of new psychoactive substances has led to the development of drug analogue laws attempting to control unspecified drug molecules not yet known to exist. Approximately 650 new psychoactive substances have been reported by 102 countries and territories since 2008 [1]. Legal control often provides the driving force for the development of new designer substances and a new drug substance must be specifically identified in order to know if it is an analogue of an already controlled drug substance or drug category. This project evaluated the dimethoxy-substituted analogues of the cathinone-type molecules and described the analytical differentiation of these regioisomeric substances using gas chromatography-mass spectrometry (GC-MS), gas chromatography-product ion mass spectrometry (GC-MS/MS) and gas chromatography-infrared spectroscopy (GC-IR).

## 2. Experimental

### 2.1. Instrumentation

GC-MS System 1 consisted of an Agilent Technologies (Santa Clara, CA) 7890A gas chromatograph and an Agilent 7683B auto injector coupled with a 5975C VL Agilent mass selective detector. The mass spectral scan rate was 2.86 scans/s. The GC was operated in splitless injection mode with a helium (ultra-high purity, grade 5, 99.999%) flow rate of 0.7 mL/min and the injection volume was 1 uL. The MS was operated in the electron ionization (EI) mode with an ionization voltage of 70 eV and a source temperature of 230 °C. The GC injector was maintained at 250 °C and the transfer line at 280 °C. The GC-MS chromatographic separations for the six intermediate regioisomeric dimethoxyphenylketones and the six regioisomeric dimethoxyphenylaminoketones (Figs. 2 and 3) were carried out on a column (30 m×0.25 mm i.d.) coated with 0.25 µm film of midpolarity Crossbond® silarylene phase similar to 50% phenyl, 50% dimethylpolysiloxane (Rxi®-17Sil MS) purchased from Restek Corporation (Bellefonte, PA). The temperature program consisted of an initial hold at 70 °C for 1.0 min, ramped up to 250 °C at a rate of 30 °C/min followed by a hold at 250 °C for 15 min.

GC-MS System 2 consisted of an Agilent Technologies (Santa Clara, CA) 7890A gas chromatograph and an Agilent 7683B auto injector coupled with a 240 Agilent Ion Trap mass spectrometer (MS/MS). The mass spectral scan rate was 2.86 scans/s. The GC was operated in splitless injection mode with a helium (ultra-high purity, grade 5, 99.999%) flow rate of 0.7 mL/min. The column head pressure was 10 psi and the injection volume was 1 uL. The MS was operated in the electron ionization (EI) mode using an ionization voltage of 70 eV and a source temperature of 230 °C. The scan type used was the Automated Method Development function (AMD) and the optimum MS/MS excitation amplitude was 1.20 V. The GC injector was maintained at 250 °C and the transfer line at 280 °C. The GC-CI-MS (using methanol as the reagent gas) and the GC-MS/MS studies were performed on a column (30 m×0.25 mm i.d.) coated with 0.10 µm film of Crossbond® 100% dimethylpolysiloxane (Rtx®–1) purchased from Restek Corporation (Bellefonte, PA). Chromatographic analysis was performed using a temperature program consisting of an initial hold at 70 °C for 1.0 min, ramped up to 250 °C at a rate of 30 °C/min followed by a hold at 250 °C for 15 min.

GC-IR studies were carried out on a Hewlett-Packard 6890 Series gas chromatograph and a Hewlett-Packard 7683 series auto-injector coupled with an IRD-3 detector obtained from Analytical Solutions and Providers (ASAP), Covington, Kentucky. The vapor phase infrared detector (IRD) spectra were recorded in the range of 4000 – 550 $cm^{-1}$ with a resolution of 8 $cm^{-1}$ and a scan rate 1.5 scans per second. The IRD flow cell and transfer line temperatures were maintained at 280 °C and the GC was operated in the splitless injection mode with a helium carrier gas (ultra-high purity, grade 5, 99.999%) flow rate of 0.7 mL/min and the injection volume was1 uL. The stationary phase used was a 30 m×0.25 mm i.d. capillary column coated with 0.10 µm Crossbond® with selectivity close to 5% diphenyl, 95% dimethylpolysiloxane (Rxi®–5Sil MS) purchased from Restek Corporation (Bellefonte, PA). The temperature program consisted of an initial temperature of 100 °C for 1 min, ramped up to 230 °C at a rate of 20 °C per minute followed by a hold at 230 °C for 15 min.

### 2.2. Synthetic methods

Precursor materials including aldehydes (2,3-, 2,4-, 2,5-, 2,6-, 3,4-, and 3,5-dimethoxybenzaldehyde), *n*-butylmagnesium chloride, bromine and pyrrolidine were purchased from Aldrich Chemical Company, Milwaukee, Wisconsin or VWR Chemical Company, Radnor, Pennsylvania. The synthetic methods needed to prepare the regioisomeric aminoketones (Compounds 1–6) in this study are well established in the chemical literature and in our laboratory [20–25]. The procedures used in this project were those reported by Kavanagh et al. [26]. These desired compounds were prepared individually from the aldehydes via a 4-step synthetic procedure. The condensation of *n*-butylmagnesium chloride (Grignard reagent) with the individual dimethoxybenzaldehyde yielded the corresponding benzyl alcohols. Oxidation of these benzyl alcohols with potassium dichromate gave the desired intermediate ketones (Compounds a-f). Alpha-bromination of the ketones at the activated methylene carbon gave the alpha-bromoketones, and subsequent displacement of the bromide ion by the nitrogen of pyrrolidine yielded the desired aminoketone final products.

The six final products were isolated by solvent extraction and purified by preparative thin layer chromatography (TLC) using a 20:80 ethyl acetate-petroleum ether solvent and AnalTech (Newark, DE) glass backed 20×20 cm plates with a 1000 µm layer of silica and an inorganic fluorescent 254 indicator.

## 3. Results and discussion

The structures of all six dimethoxypyrovalerones (DMPVs) are shown in Fig. 1. All these regioisomeric compounds have identical elemental composition and identical functional groups arranged differently within the molecule. Additionally, these six DMPV isomers likely represent regioisomerism within the major electron ionization (EI-MS) spectral fragment ions. Thus, these uniquely similar compounds are likely to yield equivalent EI-MS spectra. The six DMPV regioisomers were prepared from the six commercially available dimethoxybenzaldehydes. Condensation of each aldehyde with *n*-butyl magnesium chloride yielded the substituted benzyl alcohol which upon oxidation produced the intermediate regioisomeric dimethoxyvalerophenones. Bromination of the active methylene of the phenones followed by displacement with pyrrolidine yielded the six DMPV isomers shown in Fig. 1.

The chromatogram in Fig. 2 shows the capillary gas chromatographic separation of the intermediate dimethoxyvalerophenones. These six regioisomeric ketones elute over approximately a one-minute time window using the mid-polarity stationary phase, Rxi®–17Sil MS. The first compound to elute is 2,3-dimethoxyvalerophenone (peak a) followed by the 2,6-isomer (peak d). These two early eluting bands