Technical Note

A Rapid and Simple GC/MS Screening Method for 4-Methoxyphenol in Illicitly Prepared 4-Methoxyamphetamine (PMA)

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ABSTRACT: 4-Methoxyamphetamine (PMA), one of the less popular designer phenethylamines, has experienced a minor resurgence in recent years. A common method for illicit synthesis of PMA is via Leuckart reductive amination of 4-methoxyphenyl-2-propanone, which in turn is produced via peracid oxidation of anethole (1-methoxy-4-(1-propenyl)benzene, or *para*-propenylanisole), a major component of star anise, anise, and fennel oils. The peracid oxidation of anethole also produces 4-methoxyphenol, which can be isolated from illicitly prepared PMA via a simple and rapid procedure, and subsequently identified via GC/MS. Thus, 4-methoxyphenol is a marker compound for identification of the anethole-based production of PMA. The presented analytical methodologies represents an alternative to headspace solid-phase microextraction/mass spectral identification techniques (GC-HSPME/MS).

KEYWORDS: Anethole, Anise oil, 4-Methoxyphenol, *para*-Methoxyamphetamine, Impurity Profiling, Forensic Chemistry.

Introduction

The recreational use of non-medicinally accepted drugs is a phenomenon that mankind has been plagued with for many years. At present, the phenethylamines amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), and 3,4-methylenedioxymethamphetamine (MDMA) are particularly popular amongst members of certain social environments. Many other more unusual phenethylamines, such as 4-bromo-2,5-dimethoxy-phenethylamine, are also encountered, but much less frequently.

The production and use of unusual phenethylamines is sometimes accidental, but is often an intentional response to laws controlling illicit drugs of abuse. Most of the common phenethylamine drugs - and virtually all of the more popular ones - are illegal, which leads clandestine chemists to engage in their illicit manufacture. However, because the laws that control illicit drugs are very specific, new and/or non-regulated drugs also occasionally appear on the underground markets. As these substances are often specifically synthesized due to their non-regulated status, they are commonly known as "designer drugs". In Europe, some recent examples of such endeavors include 4-methylthioamphetamine (4-MTA) and 4-iodo-2,5-dimethoxyphenethylamine (2C-I).

4-Methoxyamphetamine (*para*-methoxyamphetamine, PMA) is a much older designer drug, which after many years of very low occurrence, has been encountered in increased frequency during the past few years - and has also been linked with a number of user deaths. However, in contrast to 4-MTA or 2C-I, PMA was already a legally restricted substance, because of its previous appearances. Due to its known high toxicity, its resurgence is somewhat surprising. PMA is usually illicitly produced via a multi-step synthesis from either anisaldehyde (4-methoxybenzadehyde) or anethole (1-methoxy-4-(1-propenyl)benzene, or *para*-propenylanisole). Anethole is a major component of star anise, anise, and fennel essential oils [1]; these oils are used in vast quantities in the food and pharmaceutical industry, and so are widely available. This latter fact makes them particularly attractive precursors for clandestine chemists.

Forensic chemists are sometimes requested to determine the precursor or the synthetic route used to prepare a seized drug preparation; this information can reveal valuable information for law enforcement agencies. Analytical information for detection of Leuckart-specific impurities in PMA has been previously reported [2,3]. However, those reports focused only on impurities that confirmed the clandestine chemist's use of the Leuckart reaction, and not on the determination of the original precursor (that is, anisaldehyde versus anethole).

We have previously reported that 4-methoxyphenol specifically derives from side reactions occurring during the peracid oxidation of anethole to *para*-methoxyphenyl-2-propanone (PMP2P) [4]. The synthesis of PMP2P from anethole is shown in Figure 1A: Anethole is reacted with performic or peracetic acid to yield the corresponding glycol. The glycol intermediate is subsequently converted to PMP2P by refluxing in a sulfuric acid/methanol mixture. The concomitant formation of 4-methoxyphenol during this reaction sequence is illustrated in Figure 1B: Oxidative cleavage of the propenyl double bond of anethole yields anisaldehyde (4-methoxybenzaldehyde), which in turn is oxidized by the peracid via a Baeyer-Villiger reaction to give *O*-formyl-4-methoxyphenol, which is further hydrolyzed to 4-methoxyphenol.

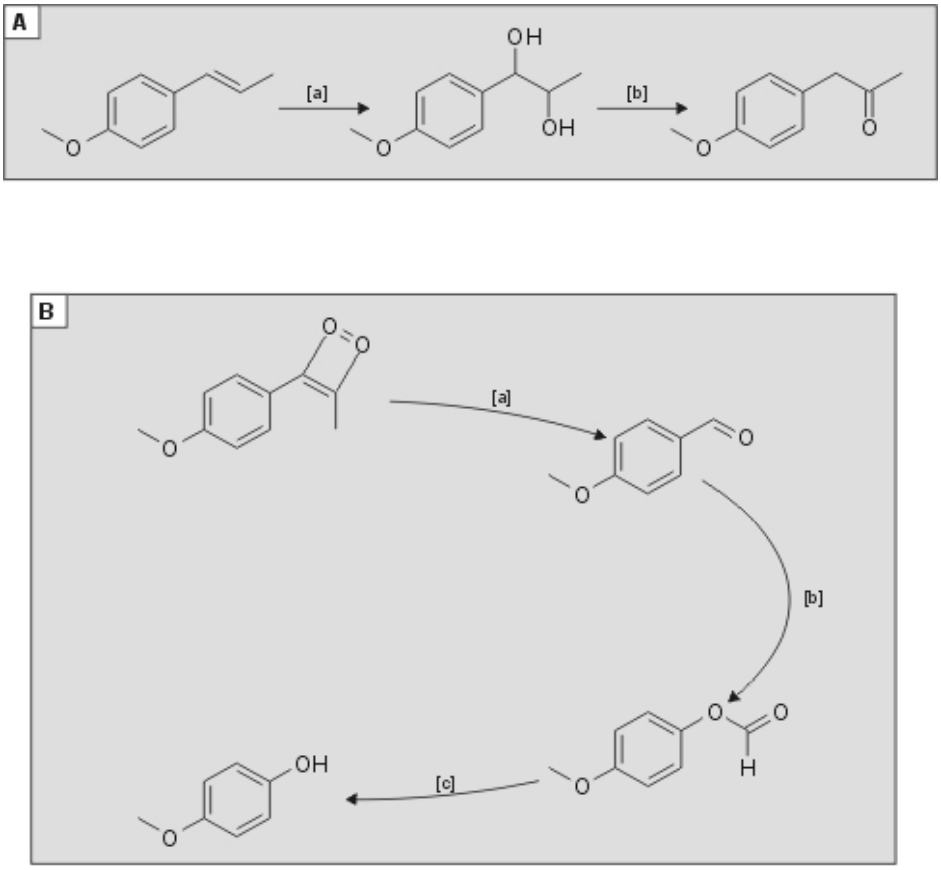

Figure 1:

A: Scheme of the peracid oxidation reaction of anethole. Anethole is reacted with a peracid ([a], typically performic or peracetic acid), after which the obtained glycol is converted to 4-methoxyphenyl-2-propanone by refluxing in a sulfuric acid/methanol mixture.

B: Overview of the 4-methoxyphenol impurity formation. The propenyl double bond of anethole is oxidatively cleaved ([a]) to yield 4-methoxybenzaldehyde. This substance is further reacted to O-formyl-4-methoxyphenol due to the presence of peracids in the reaction mixture ([b]). This compound will then be hydrolyzed to yield 4-methoxyphenol ([c]).

We have previously reported the use of headspace solid-phase microextraction - GC/MS (GC-HSPME/MS) technique for detection of 4-methoxyphenol in illicitly prepared PMA [4]; however, this methodology is not yet generally available in forensic laboratories. Herein we report a more simple and rapid technique for extraction and identification of 4-methoxyphenol.

Experimental

Chemicals

All solvents were analytical grade and were purchased from Acros Organics (Geel, Belgium). Anise oil was obtained from Taiga International NV (Breendonk-Puurs, Belgium). Unless otherwise stated, all other chemical substances were procured from Merck (Darmstadt, Germany).

Instrumentation

Gas Chromatography-Mass Spectrometry (GC/MS) analysis were run using an Agilent 6890 Plus Gas Chromatograph (GC) equipped with an Agilent 5973N Mass Selective Detector (MSD), with electronic pressure programming. For the GC, Helium was used as a carrier gas at a constant flow rate of 1.0 mL/min; the column was a 30 m x 0.25 mm x 0.25 μm VF-5MS Factor-Four capillary (Varian). Oven programming was as follows: 50˚C (held for 1 min), 35˚C/min to 100˚C, 10˚C/min to 270˚C (held for 5 min). A standard split/splitless liner was applied for liquid injections. The injector temperature was maintained at 280˚C. For liquid injections (1 μL), the apparatus was run in splitless or split mode (1:50), depending on the nature of the sample. The mass spectrometer (MS) was operated from 36 to 400 amu in electron impact (EI) mode, with an ionization energy of 70 eV. A solvent delay of 4.00 minutes was applied.

Performic Acid Oxidation of Anethole

A 250 mL round-bottomed flask was equipped with a magnetic stirbar and a thermometer, and charged with a solution of 6.0 grams of anise oil in 30 mL acetone. Performic acid solution (prepared by combining 7.0 grams of 30 % hydrogen peroxide with 25.0 grams of formic acid) was added at such a rate that the reaction mixture temperature did not exceed 38˚C. After addition of the performic acid solution, the reaction was allowed to sit for about 12 hours. The resulting mixture was poured into an equal volume of cold distilled water, then extracted with 2 x 50 mL dichloromethane. The yellow organic phase was isolated and washed with 75 mL of distilled water, after which the organic phase was dried over anhydrous sodium sulfate. An aliquot of 1 μL was subsequently injected on the GC/MS.

Sample Preparation

An aliquot of a drug preparation (100 mg for powders, 75 mg for pulverized tablet) was dissolved in 5 mL 0.1 N hydrochloric acid, after which 5 mL dichloromethane was added. The mixture was vigorously shaken for about one minute, after which the organic layer was isolated and dried over anhydrous sodium sulfate. An aliquot of 1 μL was subsequently injected on the GC/MS.

Results and Discussion

4-Methoxyphenol as Specific Peracid Oxidation Marker

We have previously analyzed star anise oil, anise oil, and fennel oil (all natural sources of anethole), and determined that 4-methoxyphenol is not naturally present in any of these essential oils. In addition, there are no literature reports of 4-methoxyphenol being present in these oils. Our analysis of commercial anisaldehyde

(Merck, Acros Organics) also confirmed that 4-methoxyphenol was not present. Furthermore, production of PMA from anisaldehyde (starting with the Henry condensation route (anisaldehyde and nitroethane) used by clandestine chemists) did not produce 4-methoxyphenol at any stage. These results confirm that the presence of 4-methoxyphenol is not due to natural contamination, or produced as a synthetic by-product in the illicit synthesis of PMA from anisaldehyde.

We have previously shown that 4-methoxyphenol is a specific marker for the synthesis of PMP2P via peracid oxidation of anethole [4]. In the present study, we repeated the performic acid oxidation of anethole, and analyzed the results by GC/MS (see Figure 2). Chromatogram 2a displays the total ion chromatogram (TIC) for 4.00 to 15.00 minutes (split injection 1:50). The inset (Chromatogram 2b) shows methyl chavicol (A), 4-methoxyphenol (B), *O*-formyl-4-methoxyphenol (C), and anisaldehyde (D). When extracting ion m/z 148, a trace of anethole is also found, as demonstrated in the extracted ion Chromatogram 2c. Methyl chavicol and anethole (both *cis* and *trans* isomers) have similar mass spectra, and identification is only possible by comparing both mass spectra and retention times.

The peaks noticed between retention time 10.00 and 13.00 are glycol derivatives (the glycol, two mono-formyl, one di-formyl, and the acetonide derivative).

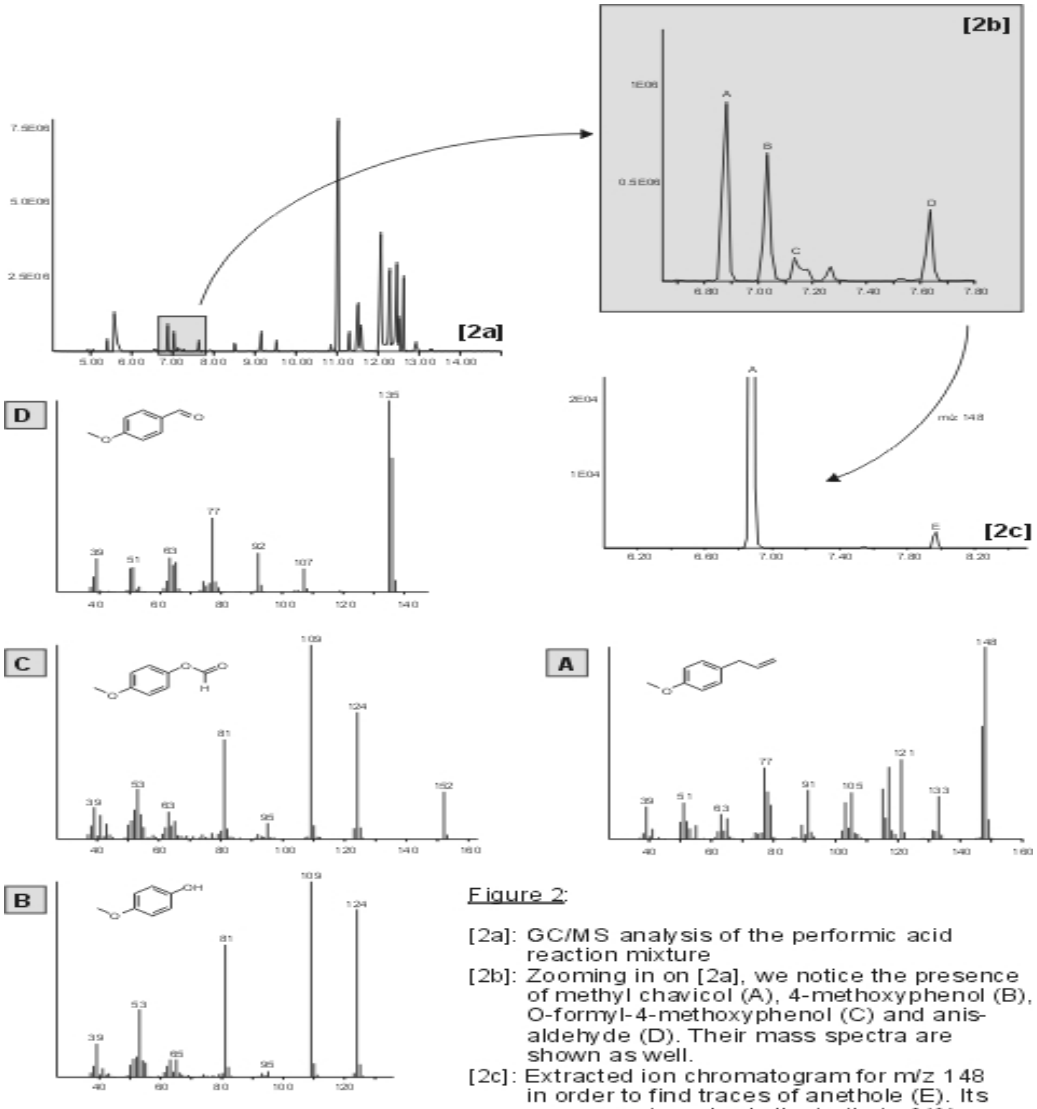

Figure 2:

[2a]: GC/MS analysis of the performic acid reaction mixture
[2b]: Zooming in on [2a], we notice the presence of methyl chavicol (A), 4-methoxyphenol (B), O-formyl-4-methoxyphenol (C) and anisaldehyde (D). Their mass spectra are shown as well.
[2c]: Extracted ion chromatogram for m/z 148 in order to find traces of anethole (E). Its mass spectrum is similar to that of (A).

Four seized samples of illicitly prepared PMA were screened for the presence of 4-methoxyphenol, using the above described procedures. The results are shown in Figure 3. Chromatogram 3a is the extract of a brownish powder, while Chromatogram 3b is the extract of a tablet (the latter tablets circulated in Belgium in 2001, and were reportedly involved in at least two deaths [5]). Chromatogram 3c is an extract of another brownish powder seized independently from 3a, while Chromatogram 3d is an extract from a powder which was contained in a capsule. In all four chromatograms, peak A is 4-methoxyphenol, while peak B is 4-methyl-5-(4-methoxyphenyl)-pyrimidine, a Leuckart reaction based impurity [4]. The results indicate that all four preparations were made using anethole (most probably as anise oil) as the original precursor.

The mass spectrum and retention time of 4-methoxyphenol gave a perfect match with a commercially obtained sample, which served as the reference standard.

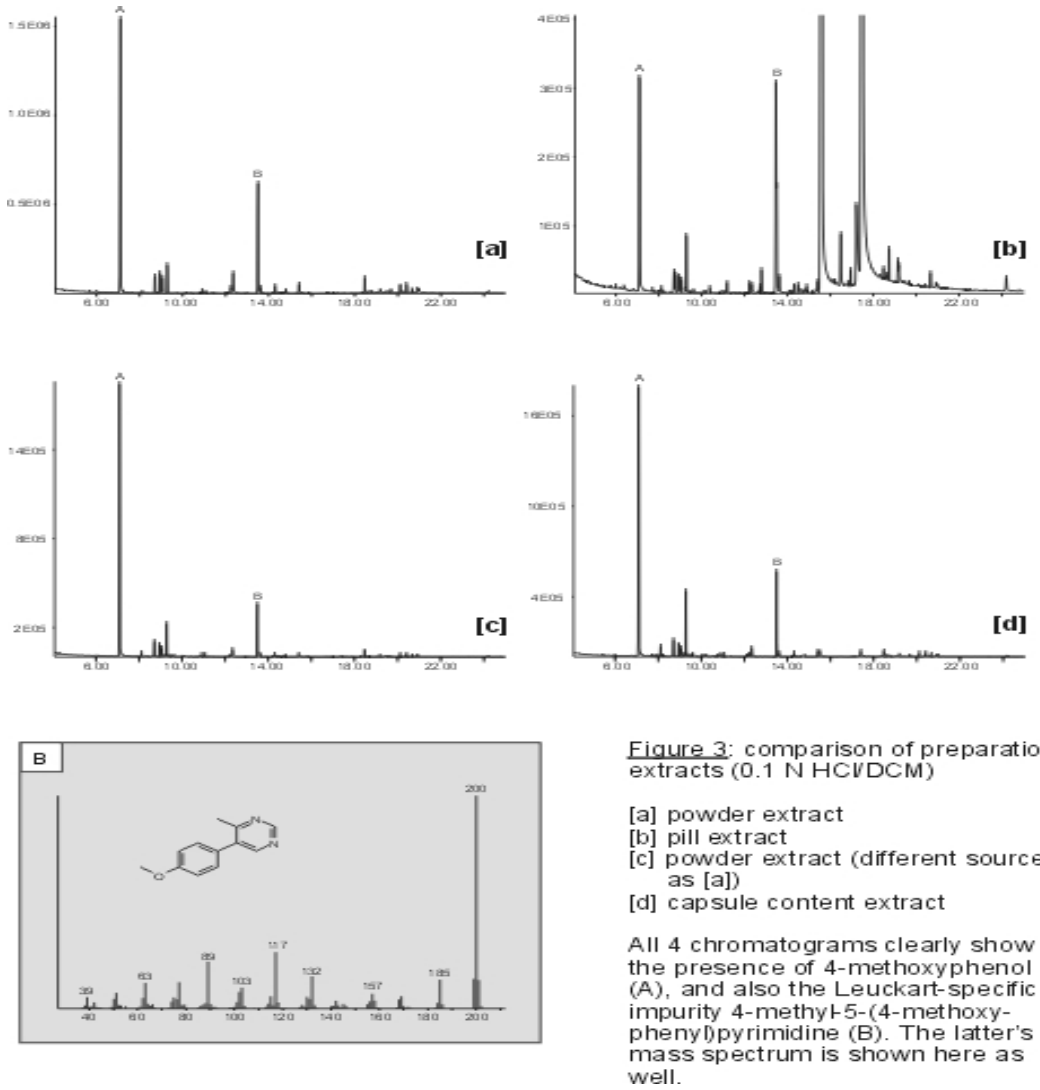

Figure 3: comparison of preparation extracts (0.1 N HCl/DCM)

[a] powder extract
[b] pill extract
[c] powder extract (different source as [a])
[d] capsule content extract

All 4 chromatograms clearly show the presence of 4-methoxyphenol (A), and also the Leuckart-specific impurity 4-methyl-5-(4-methoxy-phenyl)pyrimidine (B). The latter's mass spectrum is shown here as well.

Conclusions

4-Methoxyphenol is a synthetic by-product formed during the peracid oxidation of anethole, and thus serves as a marker compound for PMA prepared using anethole as the original precursor. The presented extraction and identification techniques are rapid and simple. Based on our work, 4-methoxyphenol would *probably* not be

present if the clandestine chemist performed a distillation to purify the intermediate 4-methoxyphenyl-2-propanone; however, few clandestine chemists perform such steps. In this study, the four illicitly prepared samples of PMA contained impurities from both the peracid oxidation and the Leuckart reductive amination reaction steps.

References

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