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Distinguishing drug isomers in the forensic laboratory: GC–VUV in addition to GC–MS for orthogonal selectivity and the use of library match scores as a new source of information

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**Abstract**

Currently, forensic drug experts are facing chemical identification challenges with the increasing number of new isomeric forms of psychoactive substances occurring in case samples. Very similar mass spectra for these substances could easily result in misidentification using the regular GC–MS screening methods in combination with colorimetric testing in forensic laboratories. Building on recent work from other groups, this study demonstrates that GC–VUV is a powerful technique for drug isomer differentiation, showing reproducible and discriminating spectra for aromatic ring-isomers. MS and VUV show complementary selectivity as VUV spectra are ring-position specific whereas MS spectra are characteristic for the amine moieties of the molecule. VUV spectra are very reproducible showing less than 0.1% deviation in library match scores and therefore small spectral differences suffice to confidently distinguish isomers. In comparison, MS match scores gave over 10% deviation and showed significant overlap in match score ranges for several isomers. This poses a risk for false positive identifications when assigning compounds based on retention time and GC–MS mass spectrum. A strategy was developed, based on Kernel Density Estimations of match scores, to construct Receiver Operating Characteristic (ROC) curves and estimate likelihood ratios (LR values) with respect to the chemical differentiation of drug related isomers. This approach, and the added value of GC–VUV is demonstrated with the chemical analysis of several samples from drug case work from the Amsterdam area involving both compounds listed in Dutch drug legislation (3,4-MDMA; 3,4-MDA; 4-MMC; 4-MEC and 4-FA) as well as their unlisted and thus uncontrolled isomers (2,3-MDMA; 2,3-MDA; 2- and 3-MMC; 2- and 3-MEC and 2- and 3-FA).

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1. Introduction

For over a decade the global illicit drug market has seen an exponential growth of so-called New Psychoactive Substances (NPS), both in amount and diversity [1]. In most cases these are derivatives of more traditional drugs with variations in substitution pattern leading to a large group of closely related chemical structures [2]. For amphetamine- and cathinone-type drugs an important emerging NPS class are ring-isomeric forms as 2-, 3- and 4-fluoroamphetamine as well as 2-, 3- and 4-methylmethcathinone.

From a legal point-of-view, correct identification of individual NPS isomeric forms is of increasing concern. In many countries, including The Netherlands, illicit-drug legislation is based on lists of banned substances [3,4]. The historical basis for these lists dates back to the United Nations Convention on Narcotic Drugs [5] and the United Nations Convention on Psychotropic Substances [6]. Associated convention reports denote multiple schedules of individually listed drugs. When a new psychoactive substance enters the drugs-of-abuse market, countries can control it by adding the compound to the banned substances list via an amendment. Reasons to initiate this are increased illicit use and associated public health concerns. Once a new substance from a
given NPS class is legally controlled this often results in a decrease in use and popularity. At the same time, however, this fuels a perpetual cycle in which new substituted or isomeric forms related to the banned substance become available on the internet and in so-called ‘smart shops’. In recent years several NPS were placed on list 1 of the Dutch Opium Act that have isomers that are not controlled but also exist in the drugs of abuse market. In 2012 methedrone (4-methylmethcathinone) was controlled by classifying it as a ‘List 1’ substance, whereas 2- and 3-methylmethcathinone are still uncontrolled. In 2017, 4-fluoroamphetamine became a listed substance, while 2- and 3-fluoroamphetamine remained uncontrolled. In addition, 4-methylcathinone (4-MEC) was added to ‘List 1’ in 2018, leaving 2-MEC and 3-MEC uncontrolled.

A legal alternative to a list-based approach for NPS is a total prohibition of psychoactive substances not specifically approved for use by individuals. This legal approach is used in New Zealand [7]. A strategy like this, however, brings other challenges as many common medicines, dietary supplements and herbal remedies are potentially prohibited. A negative-listing system excluding specific substances from the scope of the act, therefore, needs to be established. Interestingly, at the time that this manuscript was written and submitted, the Dutch government made a public announcement stating that new legislation is in preparation to move from a list-based approach to a class/group-based approach to improve the legal framework to combat the growing designer drug production and trade in the Netherlands.

Procedures used in high volume forensic laboratories dealing with drug identification are based on a combination of analytical techniques. International guidelines classify these techniques in three categories based on their discriminating power (i.e. selectivity). As a minimum for identification a ‘category A’ technique like mass spectrometry must be used in addition to one other technique [8]. Routine strategies for drug identification include colorimetric tests followed by a GC–MS targeted screening or, in case of relatively pure substances, stand-alone spectroscopic analysis like FTIR [9,10]. GC–MS based techniques in principle provide sufficient selectivity when the encountered drug substances differ in molecular mass and functional groups, thereby yielding specific differences in retention time and electron impact (EI) mass spectrum. However, amphetamine- and cathinone-type drugs often lead to ‘information deficient’ EI mass spectra as they undergo extensive fragmentation. This leads to an abundant, low m/z, ionium-ion fragment resulting from α-cleavage of the amine [11,12]. More informative m/z fragments of higher mass are only present at low intensities. In the case of ring-isomers, this phenomenon leads to highly similar mass spectra. GC retention times are also expected to show small differences as boiling points and polarity are very similar for ring-isomers. This effect will be even more profound in a broad screening method for illicit drugs as the vapor pressure of amphetamine-type and cathinone-type substances is high in comparison with the more traditional drugs. These compounds will therefore exhibit relatively short retention times leading to poor resolution. This lower discrimination power of GC–MS for ring-isomers shows the limitations of the traditional approach and brings additional challenges for the forensic chemist as this poses a potential risk for false-positive or false-negative identifications given the increasing number of NPS entering the illicit drug market.

Several strategies have been shown to be successful for efficient drug isomer identification, including techniques as cold electron ionization, leading to more informative fragmentation and enhanced molecular ion intensities [13], product ion tandem mass spectrometry [14,15], GC–MS/MS [16], derivatization techniques followed by GC–MS [17,18], UPLC-QTOF-MS [19,20], NMR [20], UPLC-fluorescence [21] and CZE [22]. However, most studies focus on cathinone-type drugs and little is reported on the discrimination of ring isomers of amphetamine-type drugs. This is remarkable as amphetamine and related drugs constitute a very significant part of the global illicit drug market and are very frequently encountered in Dutch case work.

In forensic laboratories the main strategy for dealing with the NPS challenge has been to introduce GC–IR in addition to GC–MS for robust drug isomer identifications. There are two types of GC–FTIR systems with different interfaces to enable IR characterization of compounds eluting from the GC column. One technique uses a cooled disk to trap components from the GC-column and perform subsequent FTIR analysis on the deposited solid-phase components. [23] The other approach is to directly measure FTIR spectra in the vapor phase using a gas phase IR detection cell. Vapor-phase GC–FTIR has been used for the identification of amphetamine positional isomers [24], methamphetamine ring-isomers [25] and ring-isomers of cathinone-type drugs. [26–29]. A cooled-disk GC–IR system has recently successfully been introduced as an accredited technique in forensic case work by the Netherlands Forensic Institute. Benefits include the option of enhanced sensitivity through analyte collection from consecutive injections and spectral compatibility with solid state IR libraries.

Vacuum ultraviolet (VUV) spectroscopy has recently been introduced as a new spectroscopic detection technique for GC providing spectral information that is complementary to other techniques [30]. GC–VUV is able to detect and characterize a broad range of compounds as the detector can accurately record UV spectra down to 125 nm, a region of the electromagnetic spectrum giving excitation of all (including aliphatic) chemical bonds in a molecule [31,32]. Compared to GC–IR, VUV exhibits MS-like features with respect to sensitivity, linearity, quantitative analysis and instrument costs. Although VUV spectra contain less chemical information than IR spectra and do not allow structure elucidation, compounds can be identified by spectral library matching. Applications of GC–VUV include various domains facing challenging identifications of large groups of isomeric or otherwise closely related compounds such as PCB’s in industrial mixtures [33], bacterial fatty-acid methyl esters [34,35], volatile organic compounds [36], pesticides [37] and carbohydrate identification [38]. GC–VUV can also be a very useful tool for group classification and quantification in complex sample matrices [39]. Anthony et al. demonstrated the additional selectivity of VUV spectra compared to MS for various classes of volatile organic compounds. [40,41] In the forensic field, GC–VUV was used for identification of nitrate ester explosives partly through thermally induced degradation in the VUV cell [42]. To date only two studies on the use of GC–VUV for forensic drug isomer analysis have been reported. Skultety et al. [43] successfully demonstrated the identification and differentiation of a large set of cathinone-type drugs using GC–VUV. The main focus of this study was to provide a comprehensive overview of VUV spectra of substituted cathinones. This type of NPS exhibits highly characteristic VUV spectra allowing straightforward identification of isomers. Buchalter et al. very recently reported the coupling of GC–VUV and cold EI MS for the analysis of fentanyl analogs [44]. To our knowledge no previous GC–VUV data are reported on amphetamine-type drugs.

The goal of this study was to provide and evaluate identification criteria for synthetic drug isomers based on MS- and VUV-data individually and combined. In addition to the VUV and MS spectral data, the contribution of retention time to the overall selectivity of the method was also investigated. To include retention time as an identification parameter, reference standards ideally need to be included to account for retention time shifts. When considering drug isomers, this is a growing challenge as reference standards are only available for a select set of compounds and only in small quantities at relatively high cost. Additionally, there is always the possibility to encounter a new, yet unknown, isomeric form for
which there is no reference standard available. In high-volume casework laboratories this will lead to a shift of analysis strategy from a targeted GC–MS method using reference standards to a mixed mode of targeted and untargeted screening involving different instruments and selective features and strict thresholds for robust chemical identification.

In this study, a comparison of the selectivity of EI mass spectra, VUV spectra and GC retention times to differentiate four relevant sets of NPS isomers (Fig. 1) is described. The discrimination power of each feature and combinations thereof is investigated in terms of false-positive and false-negative rates, associated Receiver Operating Characteristics (ROC curves) and evidential value (Likelihood Ratios or so-called LR values) for case studies using reference data.

The methodology and framework presented is focused on the specifically detrimental scenario in which an unknown and uncontrolled new compound is encountered in casework for the first time and is mis-identified as a listed substance. This scenario would then almost certainly lead to a wrongful conviction based on the results of the chemical analysis. With no reference data and no knowledge of the new uncontrolled isomer, the use of GC–VUV in addition to GC–MS could provide enough information to trigger an alarm on the basis of small differences in spectral match scores and retention times. Such an alarm would then initiate further

Fig. 1. Set of synthetic drug isomers selected for this study. Names underlined and in red are controlled substances in The Netherlands, the associated isomers are unlisted. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
inclusion of their molecular structure, legal status in The Netherlands and abbreviation used in this paper are given in Fig. 1. Reference materials of 2-fluoroamphetamine (2-FA); 3-fluoroamphetamine (3-FA); 4-fluoroamphetamine (4-FA); 2,3-methylendioxymethamphetamine (2,3-MDMA); 2,3-methylenedioxymethamphetamine (2,3-MDA); 2-methylmethcathinone (2-MMC); 3-methylmethcathinone (3-MMC); 4-methylmethcathinone (4-MMC); 2-methyl-ethcathinone (2-MEC); 3-methylmethcathinone (3-MEC) and 4-methylmethcathinone (4-MEC) were purchased as >98% purity HCl salts from Cayman Chemical Company (Ann Arbor, MI, USA). Reference standards 3,4-methylenedioxymethamphetamine (3,4-MDMA) and 3,4-methylenedioxymethamphetamine (3,4-MDA) were synthesized by the Amsterdam Police Laboratory. Compound standards for 5-(2-amino propyl)benzofuran (5-APB) and 6-(2-amino propyl)benzofuran (6-APB) originated from seized material that was tested in the laboratory. For 3,4-MDA; 3,4-MDMA; 5-APB and 6-APB the chemical identity was confirmed with FTIR and GC–MS.

2.2. Instrumets and settings

GC–VUV experiments were performed on a 6890 GC system from Agilent Technologies (Santa Clara, CA, USA) equipped with an Optic 3 high performance injector from GL Sciences (Eindhoven, The Netherlands) and a GC PAL autosampler from CTC Analytics (Zwingen, Switzerland). A VGA-101 vacuum ultraviolet detector from VUV Analytics (Cedar Park, TX, USA) was connected to this GC via a heated transfer tube. VUV data acquisition was performed with a VEGA Controller. For data processing and library construction and searches VUVision 3.0.1 was used.

GC–MS experiments were performed on a 7890B GC equipped with a split/splitless inlet and connected to a 5977B single quadrupole mass spectrometer, all from Agilent Technologies (Santa Clara, CA, USA). A Combi PAL autosampler from CTC Analytics was used for automatic sample introduction. Data were acquired using Masshunter GCMS Data Acquisition version B.07 and processed with MSD Chemstation Data Analysis version F.01. MS spectral deconvolution was performed with Automated Mass Spectral Deconvolution and Identification System (AMDIS) version 2.73. Mass spectral libraries were built and searched with NIST MS Search version 2.3. Match scores were returned on a scale from 0 to 1000.

On both systems the same chromatographic parameters were used, except for specific settings for the different detectors. Additionally, a higher split ratio in the injector was applied for the GC–MS due to its higher sensitivity. A volume of 1 μL of extract was injected in split mode at 300 °C with a split flow of 75 mL/min for GC–MS and 22 mL/min for GC–VUV. The liner was a deactivated glass wool split liner. The column flow was 1.1 mL/min helium in constant flow mode and separation was carried out on a ((5% phenyl)-methylpolysiloxane) HP-5MS column from Agilent Technologies of 30 m length; 0.25 mm internal diameter and 0.25 μm film thickness. The oven temperature program started at 100 °C, holding for 1.5 min, then ramping at 30 °C/min to 300 °C, no hold time, followed by 50 °C/min to a final temperature of 325 °C, holding for 2 min. The ramp to 325 °C was solely for removal of potential high boiling point contaminants that might be present in the street samples of drugs and was not needed for the chromatographic separation as all components in this study eluted well below the 300 °C.

MS settings were as follows: MS transfer line temperature: 280 °C; ionization mode: EI 70 eV; source temperature: 230 °C; quadrupole temperature: 150 °C; MS parameters: full scan acquisition from m/z 41 to m/z 462; threshold 150, scan speed 3.125 scans/sec. The mass spectrometer was routinely tuned every week using the built-in autotune program.

VUV settings were as follows: VUV transfer tube temperature: 275 °C; VUV flow cell temperature: 275 °C; make-up pressure: 0.35 psi with nitrogen gas; acquired spectral wavelength: 125 nm–430 nm at 11 ms rate with 10 averages per datapoint (i.e. 9.1 Hz). Before each run, the software collects both dark- and reference scans at 11 ms × 10 averages. Spectra were searched using the built-in library search algorithm in the VUVision software. VUV spectral match scores (R²) were returned on a scale from 0 to 1. For harmonization purposes all VUV match scores were multiplied by 1000 and thus aligned with the MS match scores.

2.3. Sample preparation

In routine illicit drug analysis, sample availability is usually not an issue as street samples contain sufficient amounts of active ingredients and are generally available in great excess of the amounts needed for analysis. For routine case samples, the Amsterdam Police Laboratory uses a validated and accredited method where 20 mg of seized case material is dissolved in 5 mL of dichloromethane leading to a theoretical concentration of 4 mg/mL for pure components. In this study 1 mg/mL solutions for all individual components were used, representing case samples containing 25% (m/v) active ingredient which is around the minimum amount routinely reported for illicit drug street samples. [45] All synthetic drugs from the phenylethyl- and cainhine-class possess a basic amine moiety. In its neutral, free-base form these components are readily soluble in organic solvents. However, street samples are mostly found in their salt form, e.g. hydrochloride, sulphate, phosphate salts, as this makes the component water soluble and leads to crystalline powders that are easy to handle and process. Commercially available standards are often also sold as hydrochloride salts. These salts are weakly soluble in organic solvents and are known to give poor peak shapes in GC. [43] Therefore, an additional step needs to be performed. In this study two different sample preparation methods were used, which both gave good peak shapes and comparable results when analyzing samples directly after preparation. The methanolic extraction was mainly used for pure substances and reference material with limited availability. The acid–base extraction was mainly applied to case–material. The method used is specified at each experiment and described in more detail below.

1) Methanolic extraction with neutralization: 0.5 mg of component was transferred into a GC vial and dissolved in 500 μL of methanol. Prior to analysis, 2 mg of sodium bicarbonate was transferred into an empty vial and an aliquot of the methanolic solution was added. The capped vial was shaken for several minutes and the sodium bicarbonate was allowed to settle at the bottom of the vial for 1 min. Part of the clear methanol was then transferred into a microvolume GC vial and injected. This neutralization strategy was described earlier by Skultety et al. [43] as a successful remedy for bad peak shapes when directly analyzing hydrochloride salts of drug compounds.
2) Acid–base extraction: 20 mg of component or sample was transferred into a test tube. 1000 μL of 1 N HCl was added and ultrasonicated for 10 min to dissolve the material. 1000 μL of 2 N NaOH was added to create the neutral basic form of the amines, directly followed by 5 mL dichloromethane. The mixture was vortexed for 10 min in a closed test tube. The lower organic layer was filtered over glass wool and Hyflo Supercell and the clean extract was transferred into a microvolume GC vial and injected. For samples with limited availability the acid–base extraction was performed in GC microvolume vials using 0.5 mg of component; 100 μL of HCl and NaOH and 0.5 mL dichloromethane.

2.4. Reference spectra collection

Reference spectra for all components were stored in spectral databases. For GC–MS a new mass spectral database was created after analysis of all individual components in 1 mg/mL dichloromethane solutions using the acid–base extraction. Each individual spectrum was stored in different ways as two separate entries. One entry as the averaged mass spectrum of all datapoints between peak widths at half maximum height and background subtraction directly before the peak basis. This was the same method used for processing of samples in all other experiments. The other entry was the deconvoluted mass spectrum obtained by AMDIS. After a library search match scores for both entries were returned. For calculations and comparison only the best spectral match (highest score) was used, however in practice both entries typically yielded highly similar match scores.

For VUV the same set of extracts were analyzed and spectra were stored in a newly created user library. The spectral range in which no absorbance was observed was excluded from the reference spectrum as the noise present in this part of the spectrum could negatively impact the match scores. For all fluoro-amphetamines the spectral range in which signal was observed was 125 nm–240 nm whereas for all other components this spectral range was 125 nm–310 nm. At the given drug concentration of 1 mg/mL no saturation of the VUV spectra was observed.

2.5. Analysis of drug isomers

Drug isomers in this study were divided into four separate groups. Components for each group were selected based on isomer class and structural similarities. Therefore, potential selectivity issues are most likely to occur in the following groups:

Group 1: 2-FA; 3-FA; 4-FA
Group 2: 2,3-MDMA; 3,4-MDMA; 2,3-MDA and 3,4-MDA
Group 3: 5-APB and 6-APB
Group 4: 2-MMC; 3-MMC; 4-MMC; 2-MEC; 3-MEC and 4-MEC

For each individual drug component at least 30 MS and VUV spectra were obtained from separate GC runs. These analyses were performed on different days during a two-month period to include effects of regular instrument fluctuations in the dataset caused by the weekly MS tune and small variations in MS vacuum and the VUV lamp output and in the state of the VUV flow cell, MS source and GC. Both the GC–MS and GC–VUV instruments were completely shut-down and restarted at least once during the two-month period. The analyzed samples comprised both dichloromethane extracts and methanolic extracts, between which no chromatographic or spectral difference was observed. Fresh samples were prepared at most two days before analysis. Each sample set was analyzed in triplicate. All spectra were searched against the dedicated spectral libraries and match scores for the component itself (true positive result) and related compounds from the same class (false positive results) were collected. For each match score, an average and standard deviation was calculated. Match score distributions for true positive matches and false positive matches were plotted as kernel density estimations (KDE) using the AMC Kernel excel add-in, version 1.0e, provided by the Royal Society of Chemistry. In all cases, the bandwidth (h-value) was manually optimized in a way that the resulting distribution plot did not show local minima that could possibly occur as a result from the discrete match score values. Receiver operating characteristic (ROC) curves were constructed from the combined KDE values for a selected substance and its related isomers by varying the match score threshold for compound identification. The Area Under the Curve (AUC) data were calculated for all ROC-curves as an indication for test performance. AUC values can range from 0.5 to 1 where a value of 0.5 means the test is uninformative whereas an AUC value of 1 represents a perfect test, i.e. a method that has a zero false positive and false negative error rate.

2.6. Case samples

Several samples of seized case material from the Forensic Laboratory of the Dutch National Police were selected to demonstrate and evaluate the performance of the methods described in this paper. These samples were previously analyzed in the ISO-17025 accredited laboratory using the routine methods for drugs identification based on colorimetric tests and GC–MS. These GC–MS results showed the presence of a certain synthetic drugs class (e.g. fluoroamphetamine) but were inconclusive regarding the specific isomeric form. All these samples were mixtures of multiple components and therefore additional FTIR experiments resulted in complex multi-component spectra. Consequently, FTIR results were inconclusive according to laboratory guidelines. The results obtained with the standard methodology thus did not provide a robust answer to the judicial question whether the material does or does not contain an illicit substance.

The twelve selected samples were tablets of different color and shape (samples #1, 3, 6, 7 and 10); colored powders (sample #2, 4, 5 and 8), liquids (sample #11 and 12) and a capsule filled with brown powder (sample #9). All samples were analyzed in six-fold on both GC–MS and GC–VUV using the same methods as the reference samples. For sample preparation the described acid–base extraction was used.

3. Results and discussion

3.1. Chromatographic analysis

All individual drug compounds (groups 1–4) gave symmetrical chromatographic peaks with an excellent MS and VUV signal-to-noise ratio. The fluoroamphetamine-isomers from ‘group 1’ were not baseline separated (Fig. 2a). Especially 3-FA and 4-FA co-eluted, while 2-FA was partially separated from the other two isomers. From the overlaid chromatograms it can clearly be seen that for the fluoroamphetamines, GC retention time alone is not able to provide sufficient isomer discrimination. It should be noted that through column selection and optimization of GC parameters full separation of the isomers most likely can be achieved. However, adapting GC methods for specific isomer pairs is not efficient when dealing with high case loads for which forensic drug laboratories typically apply broad screening methods and short run times. Substances from ‘group 2’ (the MDMA-type drug) were all baseline separated (Fig. 2b). Although, the current method, separation seems appropriate for MDMA and related components, identification on retention time in the absence of reference standards is not
recommended considering instrumental variations and the small differences in elution times of these isomers.

The APB isomers (‘group 3’) gave severe co-elution and could not be separated with the current chromatographic method. In the 6-APB chromatogram an extra peak at around 6.28 min is visible. This peak is considered to be an impurity in the 6-APB material and is further ignored as this peak is well separated from isomers of interest.

Finally, all components from the cathinone-isomers (‘group 4’) were quite well separated with only partial co-elution visible for 2-MMC, 4-MEC and 4-MMC. In practice this will not often cause a problem as the real challenge is to distinguish isomers from the same sub-type as the MMC and MEC type compounds have a different molecular mass and thus a different base peak ion in the mass spectrum. The GC–VUV overlay chromatograms of the ‘group 3’ and ‘group 4’ compounds can be found in the Supplementary Information.

In general, peaks in GC–VUV were approximately 25% broader than in GC–MS but still appropriate for analysis. The additional peak broadening is caused by the 40 µL flow cell in the VUV instrument. Make-up gas flow was optimized at 0.35 psi for maximum sensitivity while maintaining peak shape. Peak widths could be improved by increasing make-up gas flows thereby reducing residence time in the VUV flow cell, but this will be a trade-off over sensitivity.

In both GC–MS and GC–VUV analyses poor peak shapes were observed in older extracts and during longer sequences. One possible cause for this is the equilibrium between the free base form and hydrochloride-salt form in methanolic extracts affecting vaporization during injection. This peak broadening effect seemed to be more severe for the early eluting fluoroamphetamines for which replicate analysis of the same methanolic extract in a sequence showed an increase in peak broadening from 0.020 min at peak base during the first injection to 0.150 min at the fourth injection 10 h later. This peak broadening can however be reversed by re-treatment with sodium bicarbonate or delayed using the acid–base extraction and creating dichloromethane extracts. This phenomenon was not caused by chemical degradation as demonstrated by the consistency of the spectra (VUV and MS).

Another issue with peak shape consistency was related to the degradation of the cathinone samples when working on a sub-optimal system. In this case, additional chromatographic peaks were visible directly before or after the main peak of interest. This effect was observed for both the GC–MS and GC–VUV instruments and was more severe with longer residence time of the components in the injection liner (e.g. lower split flows), higher injection temperature and the use of older liners. These observations indicate that this phenomenon is related to active sites and thermal decomposition in the liner. In GC–MS these additional peaks gave a mass spectrum similar to the main peak, but with a –2 m/z shifted base peak ion. Degradation of cathinone compounds in the injector was described before by Kerrigan et al. [46] who attributed the observed degradation to enamine formation of the cathinones on active sites in the GC-system. This enamine formation explains the –2 m/z mass shift observed in the MS spectra. In our systems this degradation effect completely disappeared when replacing the liner.
In this study excellent peak shape and retention time repeatability were obtained when analyzing fresh extracts and regular cleaning or replacement of the injection liner. The average retention time and associated standard deviation for all components can be found in the Supplemental Information. Observed residual standard deviations were in the range of 0.1–0.02%. It should be noted that the data provided in this table were acquired on a single instrument thus not representing instrument-to-instrument and lab-to-lab variation.

### 3.2. Mass spectral match scores

Baseline subtracted mass spectra for the fluoroamphetamine, MDMA and MDA, APB and cathinone isomers showed a high degree of similarity within their isomeric class (spectra can be found in the Supplemental Information). These information-poor mass spectra all yield a high intensity base fragment ion at 44 m/z (primary amines); 58 m/z (methyl-aminos) or 72 m/z (ethyl-aminos), corresponding to the iminium ion resulting from the α-cleavage of the amine moiety. For the fluoromethamphetamines additional ions at much lower intensity are visible at 109 m/z originating from the fluorobenzyl cation and 83 m/z [20]. No specific discriminating ions were observed for individual isomers. Except for some small intensity differences for low abundant ions, the spectra were almost identical. The same is seen for the isomers in group 2 where 3,4-MDA and 2,3-MDA also gave quite similar mass spectra with 44 m/z as main ion fragment. The other isomeric pair, 3,4-MDMA and 2,3-MDMA both gave a 58 m/z base peak resulting from the extra methyl-group leading to a N-methyl-imine ion [47]. This illustrates that with GC–MS different isomeric sets are easily distinguished but that within such a set discrimination is much more challenging. This similarity in mass spectra for isomeric components was confirmed by the GC–MS spectral match scores for these components as listed in Table 1. For all combinations of isomers, true positives and false positives gave a match score above 900 for the fluoroamphetamines, APBs and cathinones. Only the 2,3- vs. 3,4-methylenedioxy-isomers from ‘group 2’ gave slightly lower false positive match scores, but still between 800 and 900. False positive matches for 3- vs 4-position isomers and vice versa yielded higher scores in comparison to the 2-position isomers. This effect was observed for all fluoroamphetamine, methyl-methcathinone and methyl-ethcathinone related compounds.

As the 4-isomers are controlled substances in The Netherlands, false-positive scores against this isomer are of most judicial interest. Given the substantial match scores for all isomers against the reference spectrum of the listed drug compound (indicated in red – false positive – and green – true positive – in Table 1), a forensic expert could arrive at a false positive identification which most likely would then result in a wrongful conviction. However, as indicated in Table 1, highest match scores are consistently found when the measured spectrum is compared against the correct reference spectrum. The reported standard deviations indicate that the difference in true positive vs false positive match scores could be statistically significant especially when dealing with the 2-position isomers. Although MS match scores are typically only used to produce rank lists of potential chemical compounds, the data presented in this study indicates that such data could also be used as a source of information to differentiate drug isomers. A suitable approach to utilize this information is discussed in more detail in paragraph 3.4.

### 3.3. VUV spectral match scores

Normalized VUV spectra of the ‘group 1’ (fluoroamphetamines) and ‘group 2’ (MDA and MDMA) drugs isomers are shown in Fig. 3. The spectra for the fluoroamphetamine isomers show a high degree of similarity whereas the VUV spectra of the MDA and MDMA compounds show clear differences for the 2,3 vs the 3,4-isomers. Interestingly, the VUV spectra for 2,3-MDA and 2,3-MDMA and for 3,4-MDA and 3,4-MDMA are almost identical illustrating the orthogonal selectivity of GC–VUV with respect to GC–MS. Combining these techniques provides a means to confidently identify MDA and MDMA isomers.

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<td>GC–MS match scores of selected components with the correct reference spectrum (true positives, in bold) and that of related compounds (false positives), average scores with standard deviation. In red (false positive) and green (true positive), match scores against the reference spectrum of the listed isomer.</td>
</tr>
<tr>
<td>GC–MS (n=30)</td>
</tr>
<tr>
<td>2-FA</td>
</tr>
<tr>
<td>3-FA</td>
</tr>
<tr>
<td>4-FA</td>
</tr>
<tr>
<td>GC–MS (n=30)</td>
</tr>
<tr>
<td>3,4-MDMA</td>
</tr>
<tr>
<td>2,3-MDMA</td>
</tr>
<tr>
<td>3,4-MDA</td>
</tr>
<tr>
<td>2,3-MDA</td>
</tr>
<tr>
<td>GC–MS (n=30)</td>
</tr>
<tr>
<td>5-APB</td>
</tr>
<tr>
<td>6-APB</td>
</tr>
<tr>
<td>GC–MS (n=30)</td>
</tr>
<tr>
<td>3,4-MMC</td>
</tr>
<tr>
<td>3,4-MMC</td>
</tr>
<tr>
<td>3,4-MMC</td>
</tr>
<tr>
<td>GC–MS (n=33)</td>
</tr>
<tr>
<td>2,3-MEC</td>
</tr>
<tr>
<td>3,4-MEC</td>
</tr>
<tr>
<td>4,5-MEC</td>
</tr>
</tbody>
</table>
For both the APB and cathinone isomers very discriminating VUV spectra were observed (spectral details can be found in the Supplemental Information). This finding is in line with an earlier report on cathinones showing different spectra for ring-isomers of the MMC class. [43] The patterns are remarkably more characteristic and discriminative than the fluoroamphetamine isomer spectra. A possible explanation is the presence of the carbonyl-group in the cathinones leading to a larger conjugated system with delocalized $\pi$-electrons giving rise to specific VUV-absorption of which the intensity and wavelength is influenced by the position of the substituents. The presence of a fluoro-group instead of a methyl-group on the fluoroamphetamines is not expected to ‘quench’ the spectral variation, as Skultety et al. [43] showed similar spectral features for fluoromethcathinone species.

Similar to the ‘group 2’ components, the complementary character of VUV versus MS is also seen for the cathinones. Compound sets with a variable ring configuration show distinctly different VUV spectra whereas compounds with a comparable aromatic ring configuration but different mass result in very similar VUV spectra. In contrast, MS spectra of cathinone isomers of the same mass but different aromatic ring geometry are almost indistinguishable whereas compounds with a mass difference are easily differentiated with MS even if they share the same ring structure. Combining GC–MS and GC–VUV thus provides a very powerful toolbox for the chemical identification of illicit drugs.

The qualitative observations described above were confirmed by the VUV spectral match scores presented in Table 2. Although spectral comparison algorithms between the MS and VUV differ and absolute match scores cannot be directly compared, a similar approach and analysis can be undertaken. In general, high match scores are reported for VUV spectral comparisons, even visually different VUV spectra often lead to modified match scores over 900. However, VUV spectra and VUV spectral match scores are much more robust and repeatable, leading to very low absolute and relative standard deviations in comparison to the MS findings. As a result, small differences in VUV spectra of drug isomers seem to be statistically more significant than observed variations in the associated mass spectra. This effect is clearly demonstrated when considering the VUV match scores of the fluoroamphetamine isomers. Although the spectra exhibit very similar lambda maxes and overall shapes, the isomers can nevertheless be differentiated easily on the basis of the match score data, owing to the spectral cross-over points (Table 2 and Fig. 3a). Because of the very high and
Table 2
GC–VUV spectral match scores of selected components with the correct reference spectrum (true positives, in bold) and that of related compounds (false positives), average scores with standard deviation. Software match scores are multiplied by 1000 for readability and comparison. In red (false positive) and green (true positive): match scores against the reference spectrum of the listed isomer.

<table>
<thead>
<tr>
<th>GC-VUV (n=30)</th>
<th>match 2-FA</th>
<th>match 3-FA</th>
<th>match 4-FA</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-FA</td>
<td>1000 ± 0.1</td>
<td>990 ± 0.4</td>
<td>995 ± 0.2</td>
</tr>
<tr>
<td>3-FA</td>
<td>992 ± 0.4</td>
<td>1000 ± 0.1</td>
<td>990 ± 0.3</td>
</tr>
<tr>
<td>4-FA</td>
<td>995 ± 0.1</td>
<td>985 ± 0.4</td>
<td>1000 ± 0.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GC-VUV (n=30)</th>
<th>match 3,4-MDMA</th>
<th>match 2,3-MDMA</th>
<th>match 3,4-MDA</th>
<th>match 2,3-MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,4-MDMA</td>
<td>1000 ± 0.1</td>
<td>956 ± 1.8</td>
<td>993 ± 0.2</td>
<td>949 ± 1.9</td>
</tr>
<tr>
<td>2,3-MDMA</td>
<td>985 ± 0.9</td>
<td>1000 ± 0.1</td>
<td>955 ± 0.8</td>
<td>996 ± 0.3</td>
</tr>
<tr>
<td>3,4-MDA</td>
<td>998 ± 0.2</td>
<td>953 ± 1.7</td>
<td>1000 ± 0.2</td>
<td>949 ± 2.0</td>
</tr>
<tr>
<td>2,3-MDA</td>
<td>953 ± 1.1</td>
<td>972 ± 1.4</td>
<td>1000 ± 0.1</td>
<td>1000 ± 0.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GC-VUV (n=30)</th>
<th>match 5,APB</th>
<th>match 6,APB</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-APB</td>
<td>1000 ± 0.1</td>
<td>985 ± 0.3</td>
</tr>
<tr>
<td>6-APB</td>
<td>985 ± 0.4</td>
<td>1000 ± 0.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GC-VUV (n=6)</th>
<th>match 2,MMC</th>
<th>match 3,MMC</th>
<th>match 4,MMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,MMC</td>
<td>1000 ± 0.1</td>
<td>918 ± 1.1</td>
<td>938 ± 0.7</td>
</tr>
<tr>
<td>3,MMC</td>
<td>915 ± 0.5</td>
<td>1000 ± 0.1</td>
<td>963 ± 0.4</td>
</tr>
<tr>
<td>4,MMC</td>
<td>944 ± 0.4</td>
<td>963 ± 0.2</td>
<td>1000 ± 0.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GC-VUV (n=6)</th>
<th>match 2,MIC</th>
<th>match 3,MIC</th>
<th>match 4,MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,MIC</td>
<td>1000 ± 0.1</td>
<td>926 ± 0.5</td>
<td>952 ± 0.4</td>
</tr>
<tr>
<td>3,MIC</td>
<td>925 ± 0.5</td>
<td>1000 ± 0.1</td>
<td>968 ± 0.3</td>
</tr>
<tr>
<td>4,MIC</td>
<td>950 ± 0.3</td>
<td>964 ± 0.3</td>
<td>1000 ± 0.1</td>
</tr>
</tbody>
</table>

consistent true positive match scores a threshold value could be applied to confirm the presence of the listed substance 4-FA and to provide a warning on the potential presence of an associated isomer even if the compound in the sample is unknown and not in the spectral library. A more structural approach in using MS and VUV match scores as a source of information to discriminate drug isomers is presented in the next paragraph. Finally, it should be noted that these results only provide insight with respect to repeatability and not reproducibility as a single GC–VUV instrument was used in this study.

3.4. Distribution of match scores and retention times

Based on the GC–MS and GC–VUV findings described in the previous paragraphs, the discriminating power of the individual and combined features GC retention time, MS match score and VUV match score was further explored. It is important to recognize that these features are now exclusively being considered to differentiate between isomer pairs according to the following generic sets of hypotheses:

\[ H_p \] (prosecution hypothesis):
‘Sample X contains the listed compound Y, and not one of its isomers’

\[ H_d \] (defense hypothesis):
‘Sample X contains the unlisted compound Z, an isomer of compound Y, and no other isomers including compound Y’

The difference should be noted with respect to considering any possible chemical compound as is typically the starting point for any illicit drug analysis. Additionally, the defense hypothesis only considers one and not all potential isomers. So for the ‘group 1’ and ‘group 4’ compounds two hypothesis pairs need to be considered, e.g. 4-FA vs 3-FA and 4-FA vs 2-FA. In this set the differentiation between 3-FA vs 2-FA is not relevant from a legal perspective as both compounds are ‘unlisted’. Library match scores should also be generated versus the reference spectrum for the listed compound, in this case 4-FA.

Receiver Operating Characteristics (ROC) constitutes a useful approach to express the discrimination power of the individual features. The well-known ROC curve is constructed by plotting the true positive rate (sensitivity) as function of the false positive rate (1 – specificity) for variable threshold values of a given test method. To be able to estimate these rates, true positive and false positive probability distributions need to be established from the data generated for the drug isomer standards. For retention time, the variation is expected to show a Gaussian distribution (in line with the peak shape). Therefore, the averages and standard deviation values listed in Table S1 in the Supplemental Information can directly be used to plot the normal probability density functions. For both the MS and VUV match scores the assumption of a Gaussian distribution is invalid as these match scores have a discrete maximum value and the characteristics of the matching algorithm are unknown. Therefore, the distribution of match scores was approximated using a kernel density estimation (KDE). To this end, at least 30 datapoints from individual replicate analyses were acquired. Fig. 4 shows the KDE match score distribution plots for the fluoroamphetamines for both MS and VUV match scores against the 4-FA reference spectra (Additional information could possibly be obtained by studying the match scores against all known isomer library spectra which could be an interesting topic for future studies). The impressive discrimination power of VUV spectroscopy already expressed via the data in Table 2 is visualized in the KDE plots. Despite the high spectral similarity of the fluoroamphetamines the true positive (4-FA vs 4-FA) and false positive (3-FA vs 4-FA and 2-FA vs 4-FA) distributions are completely separated. In contrast, the MS match score distributions show significant overlap especially for 3-FA/4-FA. The corresponding ROC curves are depicted in Fig. 5. With AUC (Area Under the Curve) values of 1, GC–VUV represents the ideal test method combining a 100% true positive rate with a 0% false positive rate for 4-FA isomer identification. The worst performance
was obtained for the MS match scores of 4- vs. 3-fluoroamphetamine with an AUC value of 0.79. Routine casework methods are based on colorimetric testing in combination with GC–MS. Clearly the use of these methods is insufficient to discriminate between 4-FA as a banned substance and 3-FA as its unlisted isomer. KDE distributions and corresponding ROC curves for the other groups can be found in the Supplemental Information. The findings are consistent with the fluoroamphetamine results. The KDE match score plots from the MS data of the cathinones also show overlapping distributions indicating a significant risk for false positive identification. VUV spectra for the cathinones, on the other hand, exhibit major differences among isomers resulting in discriminating match scores with even larger differences than for the fluoroamphetamines and APBs. Indeed, all VUV based ROC-curves in the Supplemental Information also yield an ideal AUC value of 1. Because completely separated distributions were already obtained for the more challenging compounds, there was no need to calculate the KDE plots and list the ROC curves for the VUV spectra of the cathinones. The same holds true for the group 2 components as match score differences for these isomers were larger compared to the other groups and a qualitative inspection of VUV and MS spectra suffices to identify the correct MDA and MDMA isomer.

The Supplemental Information also contains GC retention time-based ROC curves for the critical, partially coeluting isomer pairs 3-FA/4-FA and 5-APB/6-APB. Retention time data from both GC–MS and GC–VUV experiments were available, but due to differences in injector type, GC model and column length fluctuations they were not comparable. We arbitrarily chose the GC–MS retention times for these distribution plots. These plots show that the retention time at peak maxima provide an additional useful feature to discriminate between these components as AUC values above 0.9 are observed in both cases. However, this complete dataset was obtained from one GC–MS instrument with a single column and in a relative short period of time involving only relatively clean samples. Due to column deterioration over time, removal of the first few centimeters of the column is common practice which will lead to systematic shifts in retention time. Variations in retention time may also be observed when using different instruments or when installing an entirely new column. Therefore, retention time-based discrimination of isomers must be used with caution and robust application will require the use of reference standards and retention time locking or calibration procedures (e.g. through Kovats indices).

3.5. Evidential value for case samples

All individual peaks detected in the chromatograms from the case samples were searched against the MS and VUV spectral databases developed for this study as well as the generally available MS spectral databases from NIST and SWGDRUG. The results of all matches and near-matches with the compounds of interest in this study are shown in Table 3. Match scores for other components or match scores well below the spectral ranges were ignored. For instance, for sample #4 only the data for the MMC isomer were taken into account, whereas the sample also contained caffeine and chloromethcathinone. Sample #2 contained ketamine and caffeine next to minor traces of methamphetamine and MDMA. Sample #3 mainly contained caffeine and piperonal although traces of fluoroamphetamine were found well below the common reporting limit of the laboratory. These samples were therefore not included in the overview presented in Table 3.

From the data in Table 3 it is clear that VUV is able to identify the correct isomeric form present in all samples as all observed match scores were included in the bandwidth of only one isomer. The MS scores were more ambiguous and in the case of FAs and MMCs, mass spectrometry was not able to clearly differentiate between the 3- and 4-isomer. All samples were analyzed in 6-fold and for these critical pairs the MS match score range for the various isomers overlapped.

An interesting result is the presence of a peak around retention time 5.11 min in samples #5 and #6. Both MS and VUV spectra gave high match scores for 6-APB, although, especially for the VUV, not within the bandwidth for identification. As 6-APB is also present in these samples, this additional peak clearly must be another compound. Given the similarity of the mass and VUV spectra, this peak probably originates from another APB isomer not included in this study, e.g. 4-APB or 7-APB. A likely candidate is 4-APB as this compound is a known impurity in 6-APB synthesis and presence in 6-APB case samples was reported earlier [48]. However, as reference standards for these isomers were not available in the laboratory, this could not be verified. The APB results were included in Table 2 to demonstrate how the GC–VUV can give a
strong alarm signal when a new, yet unknown isomer is encountered that is not included yet in the spectral reference databases.

In the forensic field a common approach to present the value of evidence is by means of likelihood ratios (i.e. LR values) under Bayes’ theorem [49,50]. In forensic drug analysis this approach is not commonly used as the very discriminating features usually provide for unambiguous identification. This will lead to absolute statements regarding chemical identity in forensic reports. However, this work shows that for isomeric drugs analyzed with GC–MS differentiation can be much more challenging. For these isomer pairs, determining the evidential value can provide important forensic insights and can assist in formulating scientifically valid expert conclusions. As a demonstration, LR values were estimated for GC–MS data considering the following generic sets of hypotheses:

\[ H_p \] (prosecution hypothesis):

‘Sample X contains the listed compound Y, and not one of its isomers’

\[ H_d \] (defense hypothesis):

‘Sample X contains a known unlisted isomer of compound Y’

The difference from the hypotheses described under 3.4 is that the defense hypothesis now considers all known uncontrolled isomers. To provide for this, probability distributions of individual unlisted (false positive) isomers were combined. As the occurrence of these unlisted compounds in Dutch case work is relatively rare, there is no reliable information on the base rate of the individual isomers. Therefore, for the false positive scenario an equal prior probability for the two unlisted isomers (e.g. 2-FA vs 3-FA) was assumed. Consequently, the combined KDE distribution was constructed by summing the equally weighted distributions of the individual isomers. LR values corresponding to the hypotheses described in this paragraph could now be estimated by taking the ratio of the probabilities of the true positive vs the combined false positive KDE distribution at a measured feature value. Combined KDE distribution plots can be found in Figure S14 of the Supplemental Information. Estimated LR values based on GC retention time and MS spectral match are shown for all case samples in Table 4. It is important to note that the LR values listed in this table are affected significantly if the actual prevalence of the unlisted isomers in the casework differs. This can lead to either reduction or increase of LR values depending whether the isomer most similar to the listed substance is more or less likely to be encountered. Because of the fully separated KDE distributions, LR values for the GC–VUV data were not included in the table. Without exception, log LR values in excess of 30 were observed demonstrating the very high evidential value that is obtained with GC–VUV for isomer differentiation. It should be noted that such high LR values are the result of extremely low probabilities for the evidence under the defense hypothesis that can only be estimated by severe extrapolation and does not represent actual data. Therefore, the table only includes the compound that was identified on the basis of the VUV spectral match. Due to the substantial variation in MS spectral match scores, LR values differ significantly for individual runs as can be seen from the minimum and maximum obtained scores for samples #1, #6 and #8. By performing the analysis in 6-fold for all samples, a reliable average value could be obtained. GC retention time and MS match score for

![Fig. 5. ROC-curves with area under curve (AUC) derived from obtained KDE data for individual fluoroamphetamine isomers spectral match scores vs 4-FA reference spectra](a: 4- vs 3-FA/MS, b: 4- vs 3-FA/VUV, c: 4- vs 2-FA/MS, d: 4- vs 2-FA/VUV).
isomers are expected to be independent features originating from different physical and chemical properties of the molecule. Combined LR values taking the retention time and MS spectrum information into account can therefore be combined by multiplication and the overall evidential value from the GC–MS analysis has been included in Table 4.

In general, GC–MS analysis of the fluoroamphetamine results in medium to strong support for \( H_p \) in positive samples. Log LR values in this case ranged between 1 and 4 for individual features and 4 and 7 for the combination. The 2-FA containing (based on VUV data) ‘negative’ sample #12 gave strong support for \( H_p \). The results indicate that for FA isomer differentiation there is substantial added value of employing GC–VUV in addition to GC–MS. LR values as low as 16 have been obtained from individual GC–MS analyses of 4-FA case samples which clearly is insufficient for stating the chemical identity of a listed compound. At first sight, the need for GC–VUV (or GC–IR) seems to be less critical for the cathinone samples (i.e. MMC and MEC) as the combined LR values are very high and consistent with GC–VUV findings. However, these high LR values are caused by the retention time data, not the MS match scores, which mainly provided weak support for the defense hypothesis. This is a highly undesirable situation because in the absence of standards it is difficult to relate retention time to chemical structure and because of the possibility of retention time shifts as discussed earlier. In case of a contradictory GC–MS result, i.e. a retention time pointing in the direction of a listed substance and a mass spectrum indicating an unlisted isomer, it will be difficult for a forensic expert to make a definite statement. Likewise, for the cathinone samples there is a need for an additional and orthogonal technique such as GC–VUV to resolve the isomer challenge. When considering forensic laboratories with a high case load analyzing thousands of samples annually on

Table 4

Probabilities and likelihood ratios calculated from GC–MS probability distribution data for the hypothesis sets formulated in paragraph 3.5 (i.e. listed compound vs multiple isomers). For samples 1, 6 and 8, LR values for minimum, average and maximum retention time and match scores from 6-fold replicates are shown. For all other samples only averages are used.

<table>
<thead>
<tr>
<th>Case sample</th>
<th>( H_p ) compound</th>
<th>GC retention time</th>
<th>MS spectral match</th>
<th>Combined LR</th>
<th>VUV result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample #1</td>
<td>4-FA</td>
<td>3.408</td>
<td>24.4</td>
<td>1.56</td>
<td>6</td>
</tr>
<tr>
<td>Sample #2</td>
<td>4-FA</td>
<td>3.409</td>
<td>54.9</td>
<td>0.280</td>
<td>196</td>
</tr>
<tr>
<td>Sample #3</td>
<td>4-FA</td>
<td>3.410</td>
<td>100</td>
<td>0.038</td>
<td>2674</td>
</tr>
<tr>
<td>Sample #4</td>
<td>4-FA</td>
<td>3.410</td>
<td>100</td>
<td>0.038</td>
<td>2674</td>
</tr>
<tr>
<td>Sample #5</td>
<td>4-FA</td>
<td>3.409</td>
<td>54.9</td>
<td>0.280</td>
<td>196</td>
</tr>
<tr>
<td>Sample #6</td>
<td>4-FA</td>
<td>3.411</td>
<td>149</td>
<td>0.004</td>
<td>10^{-4}</td>
</tr>
<tr>
<td>Sample #7</td>
<td>4-FA</td>
<td>3.360</td>
<td>&lt;10^{-30}</td>
<td>63.7</td>
<td>&lt;10^{-30}</td>
</tr>
<tr>
<td>Sample #8</td>
<td>4-FA</td>
<td>5.215</td>
<td>62.9</td>
<td>14.1</td>
<td>4.5</td>
</tr>
<tr>
<td>Sample #9</td>
<td>4-FA</td>
<td>5.216</td>
<td>70.1</td>
<td>5.00</td>
<td>14</td>
</tr>
<tr>
<td>Sample #10</td>
<td>4-FA</td>
<td>5.217</td>
<td>75.2</td>
<td>1.48</td>
<td>51</td>
</tr>
<tr>
<td>Sample #11</td>
<td>4-FA</td>
<td>5.018</td>
<td>&lt;10^{-30}</td>
<td>39.7</td>
<td>&lt;10^{-30}</td>
</tr>
<tr>
<td>Sample #12</td>
<td>4-FA</td>
<td>5.113</td>
<td>197</td>
<td>&lt;10^{-30}</td>
<td>&lt;10^{-30}</td>
</tr>
<tr>
<td>Sample #13</td>
<td>4-FA</td>
<td>5.369</td>
<td>54.0</td>
<td>&lt;10^{-30}</td>
<td>&lt;10^{-30}</td>
</tr>
<tr>
<td>Sample #14</td>
<td>4-FA</td>
<td>5.368</td>
<td>69.9</td>
<td>&lt;10^{-30}</td>
<td>&lt;10^{-30}</td>
</tr>
<tr>
<td>Sample #15</td>
<td>4-FA</td>
<td>5.367</td>
<td>296</td>
<td>&lt;10^{-30}</td>
<td>&lt;10^{-30}</td>
</tr>
</tbody>
</table>
different instruments, introducing GC–IR and/or GC–VUV in addition to GC–MS is of critical importance to deal with the NPS challenge and prevent misidentification.

Regarding the approach and results reported in paragraphs 3.4 and 3.5 a few important limitations need to be noted. Firstly, the generated LR data is of an indicative nature only, as no thorough validation has been conducted [51]. The aim is to provide insight with respect to the chemical analysis strategy that is required to robustly distinguish drug isomers. This is quite different from the robust methodology available for determining the evidential value of biometric comparisons using automated algorithms. Secondly, the possibility of isomer mixtures, i.e. the presence of multiple isomers in a given case sample, has not been considered. This could lead to co-eluting GC peaks and thus mixed VUV and MS spectra. Although such isomer mixtures rarely occur in case work, this would be an interesting topic for future research. Reduced library match scores for isomer mixtures including the listed drug could then lead to false negative outcomes. However, in this case the GC–VUV analysis would probably provide clear indications that the spectrum does not resemble any of the library entries. Spectral deconvolution using AMDIS (Automated Mass Spectral Deconvolution and Identification System) for MS spectra and the VUV deconvolution software [38] could then be considered. Deconvolution of mass spectra with AMDIS can be achieved without the need for reference spectra although a high degree of spectral similarity (as is the case for isomers) can cause complications. For GC–VUV deconvolution requires the reference spectra of all co-eluting compounds which is not the case when faced with a new isomer for the first time. Additionally, any deconvolution procedure fails for perfectly co-eluting compounds. Therefore, a sensible strategy could be to optimize the GC separation when a sample is encountered for which co-eluting drug compounds are suspected on the basis of the spectral data.

4. Conclusions

This study shows the potential of GC–VUV to provide orthogonal selectivity with respect to GC–MS analysis. VUV spectra show distinct differences for aromatic ring isomers whereas drug compounds with different amine moieties are usually easily distinguished on the basis of their mass spectra. Although for some classes of drug compounds the VUV spectra seem very similar, small differences are sufficient to confidently differentiate isomers due to the robustness and consistency of the spectral data. This has been demonstrated in this study with the analysis of fluoroamphetamine isomers 2-, 3- and 4-FA. KDE probability graphs of VUV match scores show completely separated false and true positive distributions leading to ideal receiver operating characteristics. The GC–VUV system does not require extensive parameter optimization and tuning, nor contains any moving parts and relies totally on basic spectroscopy principles following the Lambert–Beer law. Although the number of forensic, scientific studies is still limited, we nevertheless expect that GC–VUV can become a valuable addition to the existing instrumentation of forensic laboratories handling large illicit drug case loads. By constructing probability distributions of chromatographic retention times and spectral library match scores, this study demonstrates that information that is typically only utilized by the forensic chemist in a qualitative manner, can also be used to express evidential values when considering listed versus unlisted drug isomers. By constructing corresponding databases based on a large number of analyses that account for intra- and inter-instrument variation, forensic case work laboratories could exploit this source of information to further strengthen their chemical identification capabilities. When dealing with new isomers for the first time, for which no reference spectra are available in the libraries, small but significant deviations from expected match scores and retention times could even serve as an alarm to trigger additional investigation into the composition of the sample using more elaborate techniques.

CRediT authorship contribution statement


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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.forsciint.2019.109900.

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