Deliberate evasion of narcotic legislation: Trends visualized in commercial mixtures of new psychoactive substances analyzed by GC-solid deposition-FTIR

Kranenburg, R.F.; Stuyver, L.I.; de Ridder, R.; van Beek, A.; Colmsee, E.; van Asten, A.C.

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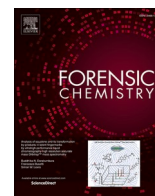Ruben F. Kranenburg [a,b,*,1], Laura I. Stuyver [a], Renee de Ridder [a], Annique van Beek [a], Erik Colmsee [a], Arian C. van Asten [b,c,2]

[a] Dutch National Police, Unit Amsterdam, Forensic Laboratory, Kabelweg 25, Amsterdam 1014 BA, The Netherlands
[b] Van 't Hoff Institute for Molecular Sciences, University of Amsterdam, PO Box 94157, Amsterdam 1090 GD, The Netherlands
[c] Co van Ledden Hulsebosch Center (CLHC), Amsterdam Center for Forensic Science and Medicine, PO Box 94157, Amsterdam 1090 GD, The Netherlands

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ABSTRACT

List-based approaches for judicial control of synthetic drugs inevitably introduce a group of legal highs that do not fall under the scope of legislation but may exhibit similar effects and associated health risks as illicit substances. Differences between controlled and uncontrolled components may be as minor as a single molecular group rearrangement. This phenomenon complicated forensic drug analysis in recent years due to both the rise of new psychoactive substances (NPS), and selectivity limitations of the workhorse gas chromatography-mass spectrometry (GC−MS) technique, especially with respect to ring-isomers. Our study demonstrates the value of GC-solid deposition-Fourier-transform infrared spectroscopy (FTIR) as a complementary technique for NPS identification in multi-drug mixtures. The instrument design using direct deposition of the GC effluent on a cryogenically cooled ZnSe-disk allows for signal enhancement of minor constituents by collecting eluting peaks of multiple GC injections. Highly diagnostic spectra were obtained for all *ortho*, *meta* and *para*-isomers of fluoroamphetamine (FA), fluoromethamphetamine (FMA), methylmethcathinone (MMC) and methylethcathinone (MEC). Combined results of GC−MS and GC-solid deposition-FTIR revealed the presence of up to 11 individual NPS mixed together in liquid samples sold as *research chemicals* or *room odorizer* in The Netherlands. Sample compositions rapidly evolved over time with recently controlled substances such as 4-fluoroamphetamine (4-FA), pentedrone, ethylone and 4-methylethcathinone (4-MEC) being replaced by uncontrolled isomers or analogues as 2-fluoroamphetamine (2-FA), 2- or 4-fluoromethamphetamine (2-FMA, 4-FMA) and dimethylone. In 12 different samples all marketed under two brand names, a total of 9 different compositions were identified in samples from 2018 and 2019.

Introduction

Over a thousand new species of recreational designer drugs have appeared on the international drugs-of-abuse market in the last decade. These so called new psychoactive substances (NPS) are often closely related to traditional illicit drugs such as MDMA, amphetamine, tryptamine or fentanyl. They often are structural analogues, positional isomers or having only minor structural modifications compared to banned substances. [1,2] The development and popularity of these substances is in many cases fueled by its uncontrolled status as a 'legal high' and these compounds could thus easily be sold to consumers via webshops or smartshops. Products are frequently labeled as a '*not for human consumption*' household chemicals to evade legislation for food supplements and pharmaceuticals. Typical labels used for NPS formulations include plant fertilizers, bath salts, incense/room odorizers or research chemicals. [3,4] Due to their novelty and large number of varieties, little is known about the potency and toxicity of these compounds. This poses a serious health risk for the users and multiple NPS-related intoxications and deaths are reported globally. [5–8] Drugs-of-abuse legislation in many countries traditionally is based on a limited list of controlled substances. A major drawback of this approach is that time-consuming legal actions need to be executed to amend the narcotic legislation

and to add new substances to the list. Meanwhile, the prospected drug substance can be freely distributed. Recently, several countries changed to a more comprehensive legal framework banning complete groups sharing a similar psychoactive molecular structure. [9] In the Netherlands, such legislation is currently also in preparation based on a combination of traditional compound lists and generalized molecular structures. [10]

The need for reliable and unambiguous identification of NPS leads to increasing challenges for forensic laboratories. When dealing with formulations containing multiple compounds dosed at different levels (*e.g.* tablets, mixtures, adulterated samples) direct spectroscopic analysis is typically not feasible. In high-volume forensic casework laboratories, full scan GC−MS analysis is the golden standard technique for routine controlled substance identification. Although suitable and recommended for the traditional illicit substances (*e.g.* cocaine, MDMA, amphetamine, heroin), the GC−MS technique has limited selectivity for unambiguous differentiation of isomeric compounds. Positional isomers of synthetic drugs can exhibit almost identical chromatographic behavior and similar fragmentation routes in electron ionization, both posing a risk for misidentification. [11–16] This can have serious adverse judicial consequences since the legal status as either a controlled substance or an unregulated designer drug can differ on minor molecular differences such as a single ring-positional rearrangement. For example, 4-fluoroamphetamine (4-FA); 3,4-methylenedioxy-*N*-ethyl-cathinone (ethylone) and 4-methylmethcathinone (4-MMC, mephedrone) currently are controlled substances in The Netherlands whereas their positional isomers 2-fluoroamphetamine (2-FA); 3,4-methylenedioxy-*N*-dimethylcathinone (dimethylone) and 3-methylmethcathinone (3-MMC) are yet uncontrolled. [17] As in forensic cases the legal outcome is typically strongly reliant on the identified compounds reported by the expert, a misidentification could easily lead to a wrongful conviction. Identification of NPS is further complicated by the continuous introduction of novel substances. Analytical reference standards might therefore be lacking or only available in small quantities. This limits the possibilities to incorporate a routine daily reference standard for retention-time-based confirmation into an analytical scheme.

Recently, various strategies have been suggested to address the analytical challenges associated with NPS identification. Since GC−MS is the standard technology available in forensic casework laboratories, approaches utilizing this equipment are preferred. Bonetti introduced the novel concept of applying multivariate statistics on mass spectra of ring-isomeric NPS to exploit the minor yet consistent differences for classification of isomeric groups. [18] Similar chemometric approaches were also successfully applied for isomeric differentiation [19,20] including calculation of the evidential value by means of likelihood ratios. [12,21] Other approaches include derivatization strategies to improve chromatographic resolution and mass spectral selectivity [21–26], or tandem mass spectrometry. [27,28] Besides these strategies that utilize conventional GC−MS instrumentation, several groups demonstrated the possibilities of ring-isomeric NPS differentiation using softer ionization techniques such as low energy electron ionization [12] or cold electron ionization. [13,29,30] In this way, only a minor technical modification on the GC−MS instrument is needed in order to produce less fragmented and more information rich mass spectra. A general drawback of all GC−MS-based approaches utilizing small but repeatable differences in retention time or mass spectrum is the need of reference standards for calibration purposes.

Spectroscopic techniques on the other hand demonstrated high selectivity for especially ring-isomeric compounds since diagnostic spectral features originate from ring-specific vibrations [31,32] and spectral libraries could be employed for identification purposes. For many years, both direct (*e.g.* attenuated total reflectance, ATR) FTIR spectroscopy and Raman spectroscopy have been and still are regarded as common analytical techniques in forensic drug testing laboratories that are capable of isomeric NPS differentiation [33–37] despite the limitations for compound mixtures due to signal overlap. Although

seized drug formulations are often being relatively pure with respect to the active ingredient, the presence of adulterants or tablet fillers such as microcrystalline cellulose frequently hamper drug identification. The combination of a chromatographic separation with spectroscopic detection is therefore often recommended for unambiguous identification of isomeric NPS in seized drug samples.

A relatively recent technique used for NPS identification is GC-Vacuum Ultraviolet (VUV) detection. Skultety et al. for the first time demonstrated its potential for isomeric designer drug differentiations. [38] Other studies demonstrated the combination and complementary nature of MS and VUV [11,13,39], the extraordinary repeatability of VUV spectra allowing for both identification and spectral deconvolution in coelutions on the basis of minimal spectral differences [40–43], and the general performance of this technique for illicit substances identification. [44]

GC coupled to infrared detection already dates back to the 1950s and has a long history with different types of interfaces and detection methods. This combination was put in place for isomeric differentiations, however practical limitations include high limits of detection, detector speed and overall ease-of-use. [45,46] Two major types of GC−IR techniques that are commercially available today are GC-vapor phase-IR and GC-solid deposition-IR. GC-vapor phase-IR analyzes substances eluting from the GC-column directly in the gas phase using a light pipe flow cell. An advantage of this technique is its relatively straightforward and non-destructive interface resulting in a robust and cost-effective detector that could be interfaced with an MS detector in a single GC instrument. In addition, vapor phase IR spectra lack the broad ~3250 cm^{-1} spectral bands originating from intermolecular hydrogen bonds and show more detailed information of the −OH stretching vibration. [45] Drawbacks include slightly broader IR absorbance bands originating from the freely rotating molecules at elevated temperatures in the flow cell and chromatographic limitations due to the maximum operatable temperature of this flow cell. [47] GC-vapor phase-IR has been used extensively for NPS identification by the Clark group [48–54] as well as others. [55–57]

Solid deposition IR, also called *condensed phase*, *solid state*, *direct deposition*, *cryotrapping*, *cryofocusing* or *cryogenic disk* IR, utilizes an interface trapping the analytes from the GC-column onto a cryogenically cooled IR-transparent surface to subsequently perform an FTIR analysis in the solid state. [58,59] Benefits of this technique include a lower limit of detection and spectra that are more comparable with conventional ATR-FTIR spectra. [45] Due to the decoupling of the GC and FTIR by using a cryogenic disc, deposited sample spots could be rescanned after analysis or the eluent of multiple separation runs could be deposited on top of each other to further increase sensitivity. [60] Only a couple of research groups have reported on the use of GC-solid deposition-IR for NPS identification in actual forensic casework. This is remarkable because many forensic laboratories have invested in this type of instrumentation to assist in NPS identification in drug analysis case work. In 2016, Angerer et al. [61] used this technique, amongst others, to elucidate the structure of an unknown synthetic cannabinoid encountered in a plant-material containing sample in an intoxication case. Solid deposition-FTIR was also used in several collaborative NPS characterization studies which reported on the usefulness of this technique for isomeric differentiations. [62–64] In 2019, Lee et al. reported a first example on the suitability of GC-solid deposition-IR to distinguish NPS by comparing library match factors of closely related substances [65] and later also reported on the feasibility of this technique for structural elucidation when combined with NMR. [66] In a recent paper, Salerno et al. extensively evaluated GC-solid deposition-IR for identification of a synthetic cannabinoid (*i.e.* JWH-018) as a proof of concept for NPS analysis. This work also evaluated the spectral differences between vapor phase IR and solid deposition IR. [47] Frison et al. demonstrated the applicability of GC-solid deposition-IR for NPS detection in biological samples by analyzing combined multiple deposits of 3-methoxy-phencyclidine from a urine sample. [60]

Previous work on GC−IR for NPS identification used the chromatographic separation solely to isolate an NPS compound either from its matrix or separate it from other drug-related compounds that are not closely related. Therefore, minimal focus was set on chromatographic resolution. Although most NPS are sold in formulations with only one active ingredient, multi-drug mixtures are sometimes also encountered in seized samples or even deliberately produced, marketed and sold as legal highs. [8,67,68] Currently, such liquid mixtures are freely available at smartshops and from several websites in The Netherlands. Besides its alleged 'not for consumption' status, accompanying information or safety data sheets also claim that these liquids contain up to 5 different non-scheduled NPS, mostly uncontrolled isomeric species of recently listed substances. This work demonstrates the added value of GC-solid deposition-IR in addition to conventional GC−MS to identify the contents of these liquids. In addition, analysis of several visually identical specimens seized between 2018 and 2020 provided insight in how formulation changes were implemented as a response to changes in the Dutch narcotic legislation in the same period.

Materials and methods

Chemicals and reagents

Reference standards of 2-methylethcathinone (2-MEC); 3-fluoromethamphetamine (3-FMA); 4-fluoromethamphetamine (4-FMA); 2-fluoroamphetamine (2-FA); 3-fluoroamphetamine (3-FA); 3,4-methylenedioxy-*N*-methcathinone (methylone); 2,3-methylenedioxy-*N*-methcathinone (2,3-methylone); 3,4-methylenedioxy-*N*-ethcathinone (ethylone); 2,3-methylenedioxy-*N*-ethcathinone (2,3-ethylone); 3,4-methylenedioxy-*N*-dimethcathinone (dimethylone), all as ≥ 98% purity HCl salts, originated from Cayman Chemical Company (Ann Arbor, MI, USA). Amphetamine sulfate (amphetamini sulfas Ph. Eur.) was purchased from Brocacef BV (Maarssen, The Netherlands). 2-methylmethcathinone (2-MMC); 3-methylmethcathinone (3-MMC); 4-methylmethcathinone (4-MMC, mephedrone); 3-methylethcathinone (3-MEC); 4-methylethcathinone (4-MEC); 2-fluoromethamphetamine (2-FMA); 4-fluoroamphetamine (4-FA); 3-fluorophenmetrazine (3-FPM); *N*-Ethylpentylone (NEP); 3-chloromethcathinone (3-CMC) and 4-chloromethcathinone (4-CMC) were provided by the Amsterdam Police Laboratory and originated from pure case materials which identities were established by the laboratory's validated qualitative analysis methods. Water (purified, Ph. Eur.); dichloromethane (analysis grade); methanol (analysis grade) and sodium hydroxide (analysis grade) were obtained from VWR International (Radnor, PA, USA). Casework samples were either materials seized by the Dutch Police or materials from which ownership was voluntarily renounced and transferred to the police by their original owners. All 21 casework samples (shown in Fig. 1) were unknown liquids stored in a closed plastic container with a commercial label. Information on the label or on the webpage of the suppliers indicated the presence of one or more uncontrolled NPS in a mixture. General brand and label information is given in the second column of Table 1. Full label descriptions can be found in Table S1 of the Supplemental Information. Actual sample volumes inside the tubes varied between 2 and 5 mL. Samples were received for initial investigation at the police laboratory between June 2018 and December 2019 and were stored at ambient temperature in the dark.

Sample preparation

For all liquid casework samples shown in Fig. 1, a 200 μL aliquot was pipetted into a glass test tube. Subsequently, 2 mL of water and 2 mL of dichloromethane were added to form 2 separate layers. Then 100 μL of a 2 N NaOH solution was added to the test tube to convert water-soluble protonated amines of NPS into their apolar free base form that will dissolve in the organic dichloromethane layer. The closed test tube is shaken for 10 mins to mix both layers, and subsequently centrifuged for

Fig. 1. All specimens of NPS-containing liquids used in this study. Letters are used for reference throughout the manuscript.

5 mins at 3500 rpm. Part of the lower dichloromethane layer is transferred into a GC-vial used for both GC-solid deposition-IR and GC−MS analysis.

For the comparative FTIR experiments described in paragraph 3.3 samples were converted to their free base form by alkaline extraction: ~50 mg of material was dissolved in 2 mL of water and 0.25 mL of 2 M NaOH solution was added. Aliquots of 1 mL DCM were subsequently added to the mixture, shaken and the bottom DCM layer transferred into an empty test tube. This procedure was repeated 5 times and all DCM fractions were combined. The DCM solution was evaporated to dryness under a stream of nitrogen. The residue was immediately analyzed by ATR-FTIR to prevent reaction with atmospheric CO_2 that could potentially lead to the formation of carbonate salts.

Instruments and settings

GC-solid deposition-IR experiments were performed on a DiscovIR-GC from Spectra Analysis (Marlborough, MA, USA) connected to a 7890B gas chromatograph from Agilent Technologies (Santa Clara, CA, USA). The GC oven was equipped with a 30 m HP-5 column (Agilent Technologies) with 0.32 mm internal diameter and a 0.25 μm film thickness. A 1 m deactivated transfer capillary with a 0.15 mm internal diameter was connected between the analytical column and the deposition tip in the DiscovIR instrument. A volume of 1 μL of a sample extract was injected using a 1:5 split at 250 °C. Helium was used as carrier gas at a 1.1 mL/min column flow (constant flow mode). The oven program started at 80 °C with a 1.5 min hold time, then rising at a 30 °C/min to 300 °C, ending with a 2 min hold time. Both the transfer line and restrictor were maintained at 280 °C, the DiscovIR was equipped with a Mercury-Cadmium-Telluride (MCT) infrared detector and a rotating ZnSe-disk on which the analytes from the GC column were trapped and subsequently analyzed in spots of 0.1 × 0.1 mm. Both the MCT and the ZnSe-disk were cooled with liquid nitrogen. The ZnSe-disk was kept at −35 °C. The temperature of the dewar cap was maintained at 35 °C. During operation, the vacuum in the chamber holding the cooled ZnSe-disk was kept at 1.3 mPa (10^{-5} Torr) and the disk rotated at a speed of 3 mm/min. The FTIR detector was set at a 700 – 4000 cm^{-1} spectral range at a 4 cm^{-1} resolution. This resulted in an acquisition rate of ~ 1.7 scans per second. The instrument configuration with the ZnSe-disk, the orifice containing the transfer line and the column tip with deposition on the cooled disk are shown in Fig. 2. The instrument was controlled and data was processed with GRAMS/AI version 9.3 with the Utilities, Data-Workup and LabFlow workbooks and SpectralID add-on installed. After analysis, multiple chromatographic plots were automatically calculated

Table 1

Summary of the substances identified in the samples. Samples sorted by type and date of receival in the laboratory. X: substance identified; XX: substance identified at higher abundance compared to other substances, m: substance identified as minor constituent; *: tentative identification due to missing reference standards or spectra, red colors are controlled substances at the date of receival; yellow colors are currently controlled substances that were uncontrolled at the date of receival.

code	name	date	2-FA	4-FA	2-FMA	4-FMA	3-MMC	3-FPM*	6-APB*	Pentedrone	4-MEC	5-EAPB*	Dimethylone	Ethylone	NEP	others
		RT MS	1.94	2.01	2.31	2.36	3.69	3.78	3.89	3.93	4.04	4.31	5.01	5.07	5.74	
		RT IR	4.36	4.39	4.68	4.71	5.85	5.88	5.98	6.05	6.14	6.35	7.05	7.08	7.70	
N	Alegria citrus	Jun/2018			X		X			X				X		
S	Alegria citrus	Sep/2018		X				X		X		X				
Q	Alegria citrus	Sep/2018	X		X	X	X									
	vendor declaration	Mar/2021	X													4-CMC, 5-MAPB
L	Alegria forest fruits	Jun/2018			X		X				X	X		X		
M	Alegria forest fruits	Jun/2018			X		X				X	X		X		
H	Alegria forest fruits	Aug/2018		m	X	XX	X	X	m			X	X			
I	Alegria forest fruits	Aug/2018		m	X	XX	X	X	m			X	X			
P	Alegria forest fruits	Sep/2018	X	m	X	XX	X	X	m			X	X	m		Ethylphenidate*
R	Alegria forest fruits	Sep/2018	X	m	X	XX	X	X	m			X	X	m		Ethylphenidate*
G	Alegria forest fruits	May/2019	X				X		m				X			
U	Alegria forest fruits, batch A01-00	Aug/2019	X				X		m				X			
F	Alegria forest fruits	Dec/2019			XX	X	X						X	X		Ethylphenidate*
	vendor declaration	Mar/2021	X				X		X							4-CMC, 4-HO-MET
J	Alegria ocean blue magic	Aug/2018		X			X	m	m			X	X		X	
K	Alegria ocean blue magic	Aug/2018		X			X	m	m			X	X		X	
	vendor declaration	Mar/2021	X				X		X							4-CMC, 2C-B-fly
O	Alegria naranja sanquina	Jun/2018			X	XX	X									
T	Alegria naranja sanquina, batch A01-002	Aug/2019					X						X			Tropacocaine*
	vendor declaration	Mar/2021					X									Tropacocaine
E	Alegria super nova party edition	Dec/2019					X				X					Tropacocaine*
	vendor declaration	Mar/2021					X									5-MAPB, 1p-LSD
A	Oli Oli Blue 5-MAPB	Dec/2019														5-MAPB*
B	Feliz X	Dec/2019					X									2C-D*
C	Oli Oli Alani 4-FMA	Dec/2019			X	X										
D	Oli Oli 3-FPM	Dec/2019						X								

and processed by the software. Unless otherwise specified, the Gram-Schmidt reconstituted plot focusing on maximum signal-to-noise throughout the chromatogram was used for visualization. All reference compounds were analyzed as 2 mg/mL solutions of the pure compound. Chromatograms of the FA, FMA, MMC and MEC isomeric sets and all reference spectra can be found in Figures S1 and S2 in the Supplemental Information. A spectral library was created from the baseline averaged spectra of the obtained peaks. In addition, 8 reference libraries containing 2.968 solid deposition FTIR spectra of both drug-related and non-drug-related substances were supplied with the instrument. Library searches were performed using a 1st derivative, correlation-based search routinely available in the software. Spectral similarity match scores ranging between 1 and 0 were returned with 0 indicating a perfect match.

For the GC−MS experiments, a 7890B GC coupled to a 5799B single quadrupole mass spectrometer was used. The laboratory's routine analytical method for illicit substance identification was applied: 1 µL of extract was injected in split mode at 300 °C using a 75 mL/min split flow

and a 1.1 mL/min column flow. The GC was operated in constant flow mode with helium as carrier gas. Separation was performed on a 15 m HP-5MS column from Agilent Technologies with 0.25 mm internal diameter and 0.25 µm film thickness. The oven started at 100 °C with a hold time of 1.5 min, the temperature was then raised at a 30 °C/min rate to 300 °C. As a final step, the oven temperature was increased to 325 °C at a 50 °C/min rate, which was maintained for 2 min. This final step was included to remove possible high boiling point substances from the column and prevent carryover. The MS was operated in full scan mode with a 41 – 462 *m/z* range.

Direct ATR-FTIR experiments were performed on a Spectrum Two FT-IR spectrometer with a room-temperature operated lithium tantalate (LiTaO$_3$) mid-infrared detector from PerkinElmer (Waltham, MA, USA) and an UATR accessory installed. Scans were recorded over a 400 to 4000 cm^{-1} wavenumber range.

For the FAs, FMAs, MMCs and MECs identification was based on the combination of retention time, IR-match and MS-match against reference standards of all possible ring-positional isomers. For pentedrone,
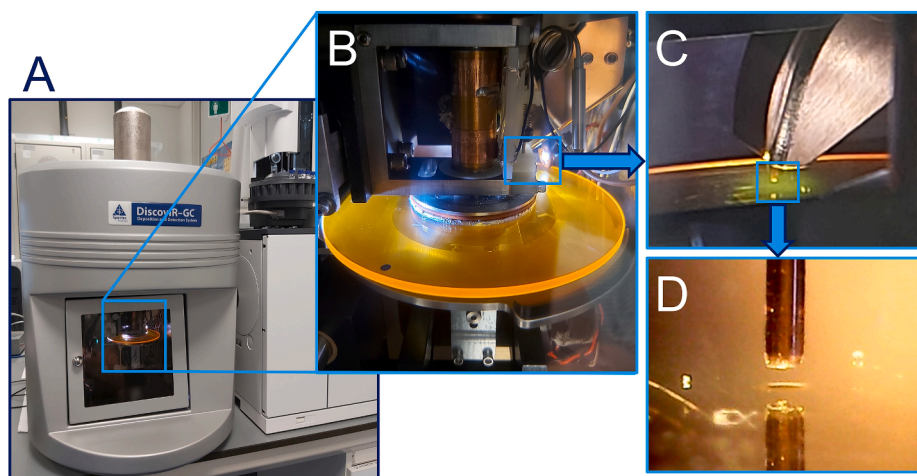

Fig. 2. Configuration of the GC-solid deposition-IR instrument. The full instrument with vacuum chamber visible through the transparent door (A), the cooled ZnSe-disk (B), the orifice with the end of the transfer line from the GC (C) and the tip of the transfer line above the surface of the ZnSe-disk with a deposition visible on the disk (D).

dimethylone, ethylone and NEP identification was also based on reference standards, however, reference material was not available for every plausible isomeric form (*e.g.* butylone) and the differentiation was accomplished by comparison with MS and IR library spectra. For 3-FPM, 6-APB and 5-EAPB, not all possible isomeric forms were present in the libraries, their identification must thus be considered as tentative.

Results and discussion

Orthogonality of spectral selectivity of MS and solid deposition-FTIR detection

Initial GC−MS analysis of the liquid casework samples revealed the presence of at least one NPS substance, although the majority of samples turned out to be mixtures of three to seven individual active ingredients. The maximum number of different NPS substances detected in a single sample was eleven. Most samples were found to contain at least one type of NPS for which different isomeric forms exist, such as fluoroamphetamines (FAs), fluoromethamphetamines (FMAs) or methylmethcathinones (MMCs). Estimated individual concentrations of these are shown in Table S2 in the Supplemental Information and were found to range between ~ 0.3 and 25 mg/mL sample (base equivalent). It must however be noted that no information on the solvents, presence of preservatives or shelf life is available and degradation over time cannot be excluded. Routine GC−MS analysis without reference standards will not unravel the precise isomeric form of these compounds due to similar EI fragmentation mechanisms leading to possibly similar retention times

and near identical mass spectra. [11,12,69] Since narcotic legislation in The Netherlands [17] differs for individual isomers of the aforementioned classes, initial GC−MS analyses were inconclusive (*e.g.* currently 4-FA is a controlled substance in The Netherlands whereas 2-FA and 3-FA are uncontrolled). Therefore, GC-solid deposition-FTIR analyses were conducted for additional selectivity. Table 1 shows the identified substances using the combined information from GC−MS and GC-solid deposition-FTIR. Figs. 3 and 4 show example chromatograms of various specimens of the *Alegria citrus* and *Alegria forest fruit* mixtures analyzed by both techniques.

In line with GC-vapor phase-FTIR [51,53,56,57], other spectroscopy-based detection techniques [11,38,39], and earlier findings from Lee et al. [65], Brandt et al. [62] and Dybek et al. [63] the GC-solid phase-FTIR spectra were found highly diagnostic for ring-positional isomers. Notable differences were observed in the 700 – 1700 cm^{-1} fingerprint area of the infrared spectrum as visible in Figs. 5 and 6. This makes GC-solid deposition-FTIR a very valuable complementary technique when combined with GC−MS, for which especially these ring isomers are most challenging. This phenomenon was clearly observed in the *Alegria forest fruit* case sample P containing (amongst others) two FA isomers, two FMA isomers and two cathinone-type isomers (shown as peaks 1–4, 6 and 7 respectively in Fig. 4). Fig. 5 shows the solid deposition-FTIR spectra of the diagnostic part of the spectrum for these peaks overlayed with reference spectra. The full range solid deposition-FTIR spectra can be found in Figure S3 of the Supplemental Information. Clear similarities were observed for the mass spectra of the adjacent peaks from isomeric pairs 1 and 2 (base peak *m/z* 44), 3 and 4 (base peak
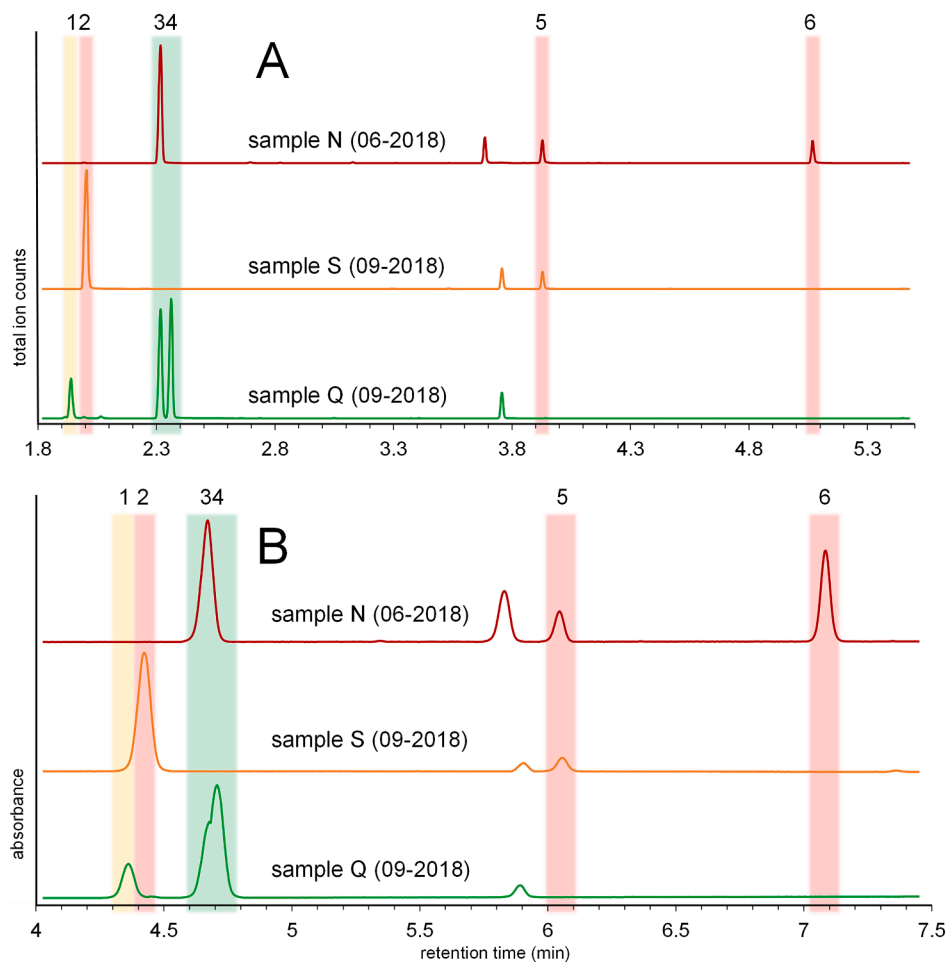

Fig. 3. GC−MS total ion chromatogram (A) and GC-solid deposition-FTIR chromatogram (B) of *Alegria citrus* samples N, S and Q. Highlighted peaks are identified as 2-FA (1), 4-FA (2), 2-FMA (3), 4-FMA (4), pentedrone (5) and ethylone (6). Red shade marks substances put under control since 2018, yellow shade marks isomers of a controlled substance that itself is currently uncontrolled, green shade marks uncontrolled isomeric NPS.

Fig. 4. GC−MS total ion chromatogram (A) and GC-solid deposition-FTIR chromatogram (B) of *Alegria forest fruit* samples L, H, P, G and F. Highlighted peaks are identified as 2-FA (1), 4-FA (2), 2-FMA (3), 4-FMA (4), 4-MEC (5), dimethylone (6) and ethylone (7). Red shade marks substances put under control since 2018, yellow shade marks isomers of a controlled substance that itself is currently uncontrolled, green shade marks uncontrolled isomeric NPS.
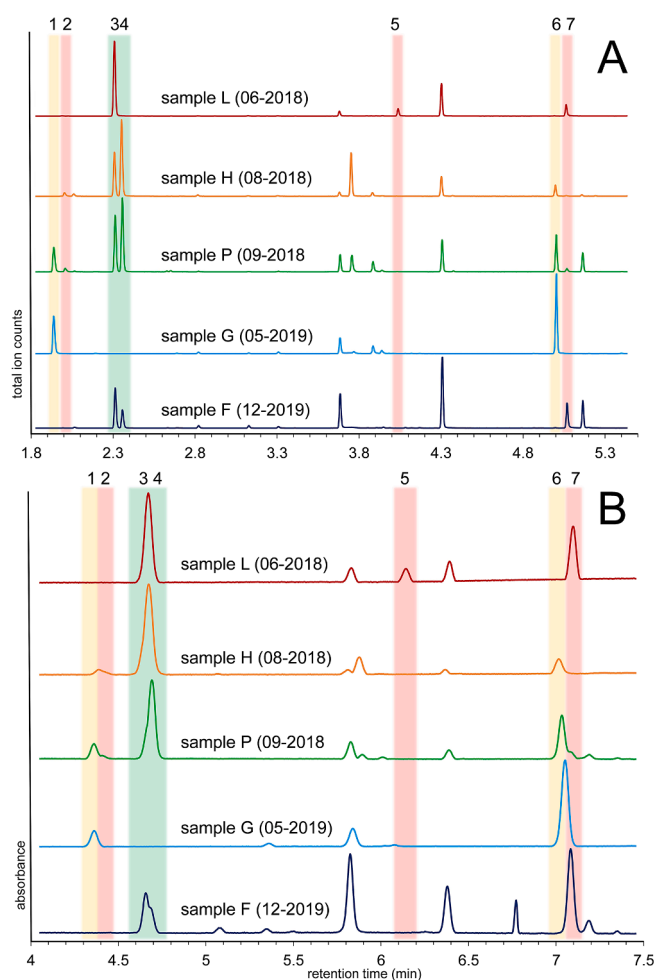
m/z 58), and 6 and 7 (base peak *m/z* 72) as can be seen in Figure S4. Nevertheless, the obtained infrared spectra (Fig. 5) showed clear differences suitable to confidently distinguish these isomer pairs. Peaks 6 and 7 were identified as dimethylone and ethylone. These substances only differ on the alkyl arrangement of the amine-group and not on their aromatic ring configuration. In this case, the most notable spectral differences were not found in the 700 – 1700 cm^{-1} fingerprint area, but in the 2700 – 3100 cm^{-1} functional group area of the infrared spectrum containing signals related to amine-group vibrations. Dimethylone (peak 6) could be distinguished from ethylone (peak 7) by two bands attributed to the C−H stretching vibration in methylated amines shown by an asterisk in Fig. 5. [70] It is however advised to always consider possible other analogues that may produce spectral similarities. For example, the isomer butylone also is a methylated secondary amine and both MS and IR spectra may show similarities with dimethylone. Butylone reference material was not available, however, in this case, distinction could be made by both MS library spectra (based on intensity difference of the *m/z* 57 fragment ion [71]) and IR library spectra (dimethylone has a peak at 774 cm^{-1}, whereas butylone has a peak at 880 cm^{-1}, false positive match scores of butylone on both dimethylone and ethylone were > 0.4 on a scale of 0 to 1).

Fig. 6 shows the solid deposition-FTIR spectra of all six FA and FMA-isomers. Clear differences between the *ortho-, meta-* and *para*-positional

isomers can be observed in the infrared fingerprint area of 700 – 1700 cm^{-1}. These bands are in most cases shared by both the corresponding FA (2,4,6) and FMA (1,3,5) isomer and are attributed to various aromatic C−H out-of-plane deformation vibrations and C−F sensitive vibrations. [70,72] Although superior for ring-positional isomer differentiation, the solid deposition-FTIR spectra also yield spectral features diagnostic for differences in the amine-moiety. Most notably, a band around 2800 cm^{-1} is only visible for the methylated compounds (Fig. 6, orange shade). This can be attributed to the −CH$_3$ symmetric stretching vibration for aliphatic amines. In addition, it could be clearly observed that for all primary amines (2,4,6) an additional band originating from the N−H stretching vibration is present around 3350 cm^{-1}. [70] Other minor differences between the primary (2,4,6) and secondary (1,3,5) amines visible at ~ 1100 cm^{-1} are related to a shift in the C−N stretching vibration. [72]

Unfortunately, not all NPS yielded highly specific solid deposition-FTIR spectra. For example, NEP and methylone -that only differ in the length of their aliphatic side-chains- shared many bands visible in the fingerprint area with minimal characteristic differences. (Figure S2-N,S and S5 in the Supplemental Information) The most notable difference is a higher relative abundance for the peaks around 3000 cm^{-1} for the longer chained *N*-ethylpentylone. This could be attributed to more abundant aliphatic C−H stretch vibrations in this substance. [70] However, the mass difference in this case makes it easy to differentiate between these two compounds on the basis of GC retention time and EI mass spectrum with GC−MS.

Match scores could be used to aid spectral identification. The software automatically calculates match scores for an unknown peak towards all library spectra and reports the highest matching spectra. In general, match scores below 0.1 were observed for true positives and as a rule of thumb, a match score below 0.05 could be used as a tentative identification criterium. However, it must be noted that these match scores are calculated by a standardized correlation algorithm applied on the first derivative of the full spectrum. In this way, all datapoints in the (transformed) spectrum are equally important. Spectra that have a lot of similarities in addition to some minor -yet important- spectral differences, may also potentially lead to a match score below 0.05. However, noise in spectra from low abundant substances was found to negatively impact the observed match scores. It is thus advised to always manually examine matches and keep focus on diagnostic spectral bands from potential analogues. In addition, other analytical information such as the mass spectrum should also correlate with the proposed identification. Table S3 gives the observed FTIR match scores from reference substances of several sets of correlated NPS. Large differences (above 0.9 on a scale of 0 to 1) were found for all positional isomers that tend to have very similar mass spectra (*e.g.* 2-FA, 3-FA and 4-FA) whereas smaller differences (between 0.12 and 0.41 on scale of 0 to 1) were observed for substances that could easily be distinguished by the base peak ion in their mass spectrum (*e.g.* 4-FA vs. 4-FMA and NEP vs. methylone). This demonstrates the added value of GC-solid deposition-FTIR when combined with GC−MS. Pure compound IR and MS spectra provide orthogonal structural information that in combination with GC retention time leads to unambiguous identification even for complex case samples containing multiple active ingredients as presented in this study. In a forensic analytical scheme for example, primary GC−MS results may identify the presence of an FA isomer, while being inconclusive on the precise isomeric form. Then subsequent GC-solid deposition-FTIR analysis assigns the correct structure from the limited set of isomeric candidates. Because of the large differences in match score, even a score between 0.2 and 0.1 -which might be the case for a low abundant peak- may suffice for this purpose. It is however recommended to always perform a subsequent visual comparison of the obtained solid deposition-FTIR spectra for identification.
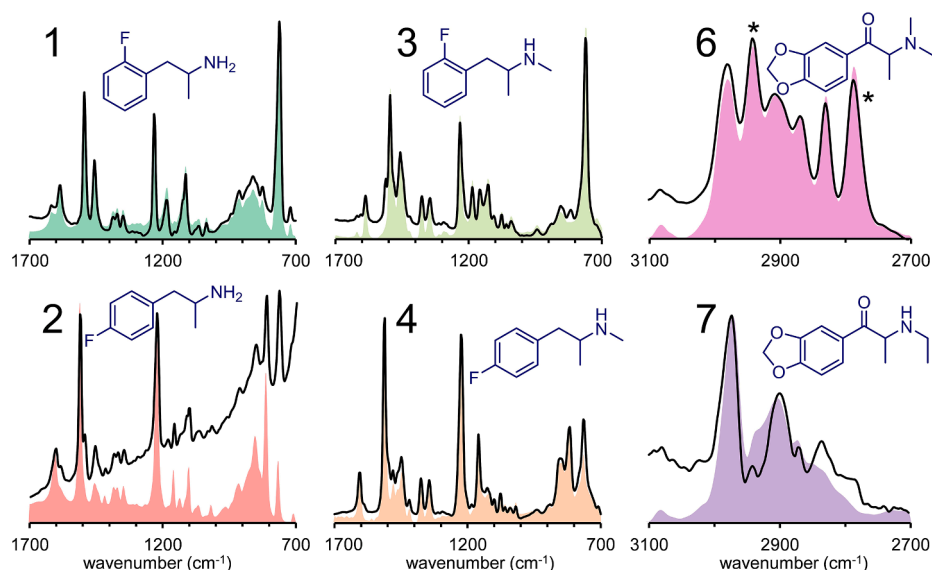
Fig. 5. Diagnostic parts of the solid deposition-FTIR spectra (black lines) from isomeric NPS detected in sample P: 2-FA (1); 4-FA (2); 2-FMA (3); 4-FMA (4); dimethylone (6); ethylone (7). Numbers correspond to peaks in Fig. 4. Colored shades are corresponding reference spectra for comparison. Asterisks indicate spectral bands diagnostic for methylated amines in the dimethylone spectrum.
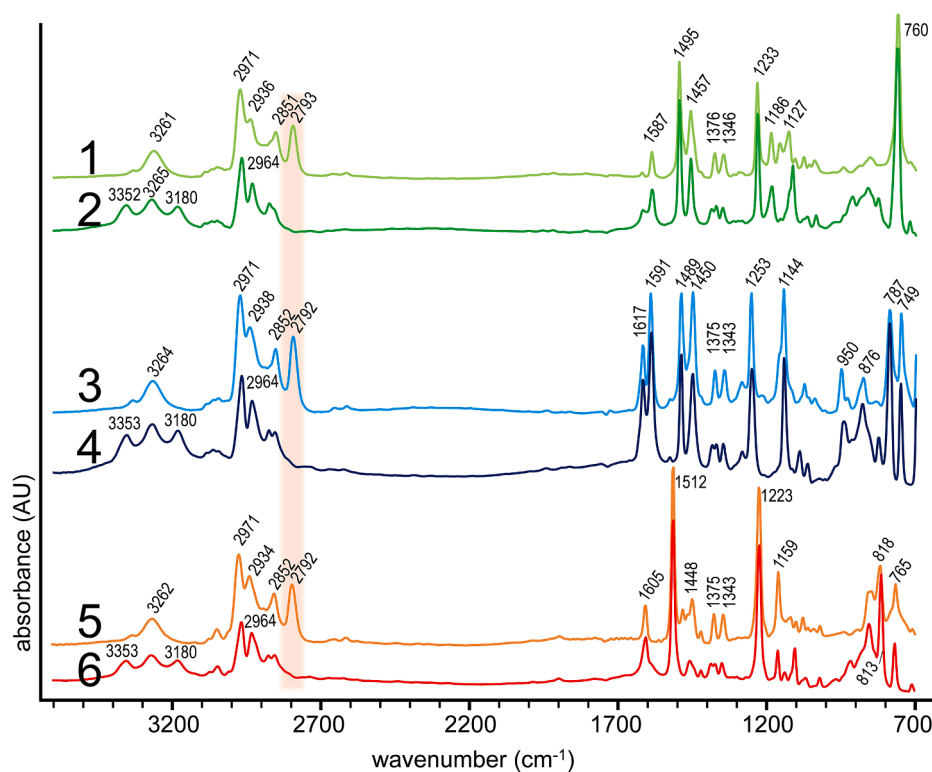

Fig. 6. Solid deposition-IR spectra of 2-FMA (1), 2-FA (2), 3-FMA (3), 3-FA (4), 4-FMA (5) and 4-FA (6). Orange shade marks the 2800 cm^{-1} band as notable difference between the FMAs and FAs. Significant differences are also apparent in the IR fingerprint area (1700–700 cm^{-1}).

Strategies to improve sensitivity and selectivity in GC-solid deposition-FTIR

In all samples, chromatographic peaks in GC-solid deposition-FTIR chromatograms were approximately 3 times broader than their corresponding peaks in GC−MS (Figs. 3 and 4). Identical results were also observed when aligning the GC methods of both instruments in such a way that similar linear velocities and chromatographic plate heights were obtained. This indicates that the design of the solid deposition interface inevitably introduces significant additional peak broadening.

Common forensic casework samples such as ecstasy tablets typically contain a well separated single main active ingredient. In this situation, a loss in chromatographic resolution is acceptable since the primary goal of the GC-separation is to isolate the active ingredient from the sample matrix. The multi-drug mixtures in this study, however, require sufficient chromatographic resolution to achieve at least partial resolution between isomeric species. Unlike VUV, in which the software holds extensive possibilities for automatic coelution detection and spectral deconvolution [43], the FTIR software does not provide such features.

For example, the coeluted peaks of 2-FMA and 4-FMA in samples H, P, F and Q (shown in Figs. 3 and 4) were initially detected as a single peak by the software. Both the non-gaussian peakshape and remarkable low match scores in this situation provided clear clues of a coelution. Manual inspection of the individual FTIR spectra over the peak and manual post-processing of a specific portion of the peak may subsequently be performed. In this way, spectra clearly matching with 2-FMA and 4-FMA (*i. e.* no visible differences and match scores of 0.0598 and 0.0104 respectively) were obtained as shown in Figure S6.

Analyte zones of interest of replicate injections can be collected on a given position to increase the absolute amount of material on the surface of the ZnSe-disk. Fig. 7 shows both the cumulative chromatographic peak and spectrum obtained for the minor (~0.3 mg/mL in sample, ~0.03 mg/mL in extract) 4-FA peak deposited on the same track of the disk from 10 replicate injections of sample H. A linear relationship between the number of stacked injections and the peak intensity can clearly be observed (Fig. 7A). The peak width, shape and retention time are surprisingly consisted, indicating a high precision and repeatability of the ZnSe-disk rotation and rewind mechanisms. Also, an improvement in visibility of the spectral bands in the fingerprint area can be seen (Fig. 7B). The match scores obtained from the stacked injections also showed an improvement due to an increase of signal-to-noise. However, the maximum benefit of stacking in spectral identification is reached after approximately 4 replicates as visible in the plot given in the Supplemental Information (Figure S7). The benefit of overlaying multiple runs is especially useful when more straightforward strategies for improved sensitivity (such as increasing the amount of sample or using a large volume injection) could not be implemented due to sample size or chromatographic limitations. In this study, this approach was used to identify the minor contribution of 4-FA (~0.3 mg/mL in sample, 0.03 mg/mL in extract) in the 2-FA-containing (~2.4 mg/mL in sample, 0.24 mg/mL in extract) sample P. Fig. 8 shows the chromatogram of the tenfold overlay (A) and the corresponding spectra (B,C). The chromatographic repeatability of this method is remarkable, given the co-elution of the both FA peaks that was already visible in the 1st injection and did not worsen after multiple deposits. The cumulative spectra yielded match scores of 0.014 and 0.226 for 2-FA and 4-FA respectively. Full spectral details can be found in Figure S8.

Another method to improve spectral quality is rescanning previously deposited peaks. In FTIR, the signal-to-noise ratio increases with the square root of the number of scans. Therefore, rewinding the ZnSe-disk to put the deposit of a certain peak in the IR beam and performing multiple FTIR scans will also increase the sensitivity. In addition, post-scanning is also possible with different settings such as spectral range or resolution. For example, the amphetamine spectrum shown in Figure S9-C was obtained by rescanning with a slightly modified scan range of 4000 – 650 cm^{-1} because amphetamine yields an interesting

spectral band at 697 cm^{-1}.

Unknown substance identification by spectral comparison between ATR and solid deposition FTIR

For the components marked with an asterisk in Table 1 no reference standards, previously recorded reference spectra or reference spectra in the solid deposition FTIR vendor libraries were available in the laboratory. Therefore, only a tentative identification based on the mass spectral match could be given. Incomplete reference libraries are a common and unavoidable situation in rapidly evolving uncontrolled environments such as the NPS market. Since IR spectra originate from vibrational modes and spectra are comparable among instruments used worldwide, the use of IR spectral libraries, especially for ATR-FTIR instruments, is common practice. The similarities and possibilities for spectral exchange between (direct) ATR-FTIR spectra and solid deposition-FTIR spectra were therefore investigated. It must be noted that NPS substances analyzed by GC are typically in their volatile neutral base form (as a result of an alkaline extraction) whereas solid samples are usually in their crystalline salt form, such as the most common hydrochloride salt. Vibrational modes and consequently the IR spectra differ for the salt and free-base form. Samples therefore need to be converted into their base form for comparison. Fig. 9 shows an example of 2-FMA analyzed as conventional hydrochloride salt in a seized sample (A), its freebase form (B) and its corresponding solid deposition FTIR spectrum (C). Notable differences were observed between the HCl salt and the freebase form (*e.g.* the 2465 cm^{-1} band originating from the amine hydrohalide N−H$^+$ strechting vibration[70]) whereas both neutral base spectra are visibly identical in the fingerprint area. For sulphate salts, such as commonly encountered amphetamine sulphate, differences between the salt and base form are much more prominent (Figure S9). ATR-FTIR spectra of several drug substances in their neutral base form were imported into the GRAMS SpectralID software and added to the spectral library. Unfortunately, match scores for these spectra against the same substance analyzed by GC-solid deposition-FTIR were always above 0.3, thus well above typical match scores observed for a true positive match. Possible explanations for this may be *i)* minor spectral differences caused by *e.g.* residual water, *ii)* differences in detection technique such as the cryogenic MCT detector in the DiscovIR versus the room-temperature lithium tantalate detector in the conventional benchtop FTIR instrument or *iii)* minor calibration offset differences between instruments. The latter could be compensated for by *e.g.* corrections against a polystyrene standard. Method transfer was outside the scope of this study, however, these spectral similarities could be potentially beneficial for structural elucidation of rare substances. Routine forensic laboratories that are only equipped with a benchtop ATR-FTIR instrument could sent (neutral base) spectra of newly
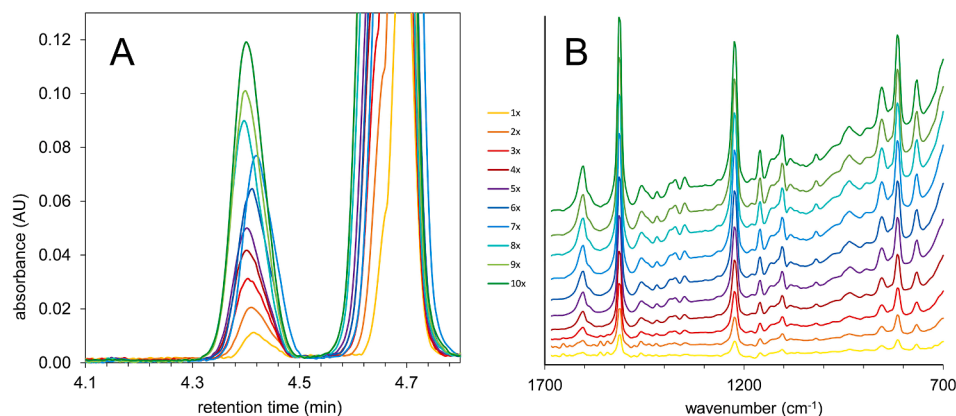

Fig. 7. The chromatographic peak of 4-FA (A) and the corresponding FTIR spectra (B) of cumulative replicate deposits of sample H on the same track of the ZnSe-disk.
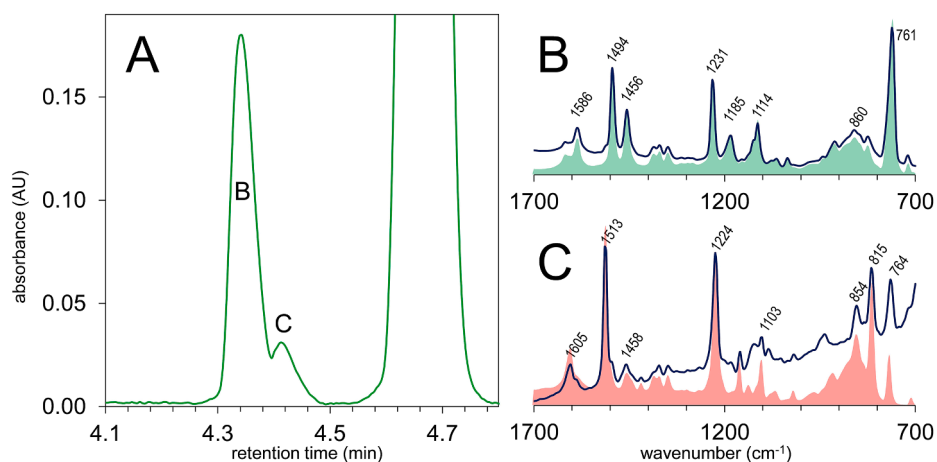
Fig. 8. Cumulative chromatogram (A) and corresponding FTIR spectra of partially coeluted peaks of 2-FA (B) and 4-FA (C) after 10 consecutive analyses of sample P. Colored shades are corresponding reference spectra for comparison.
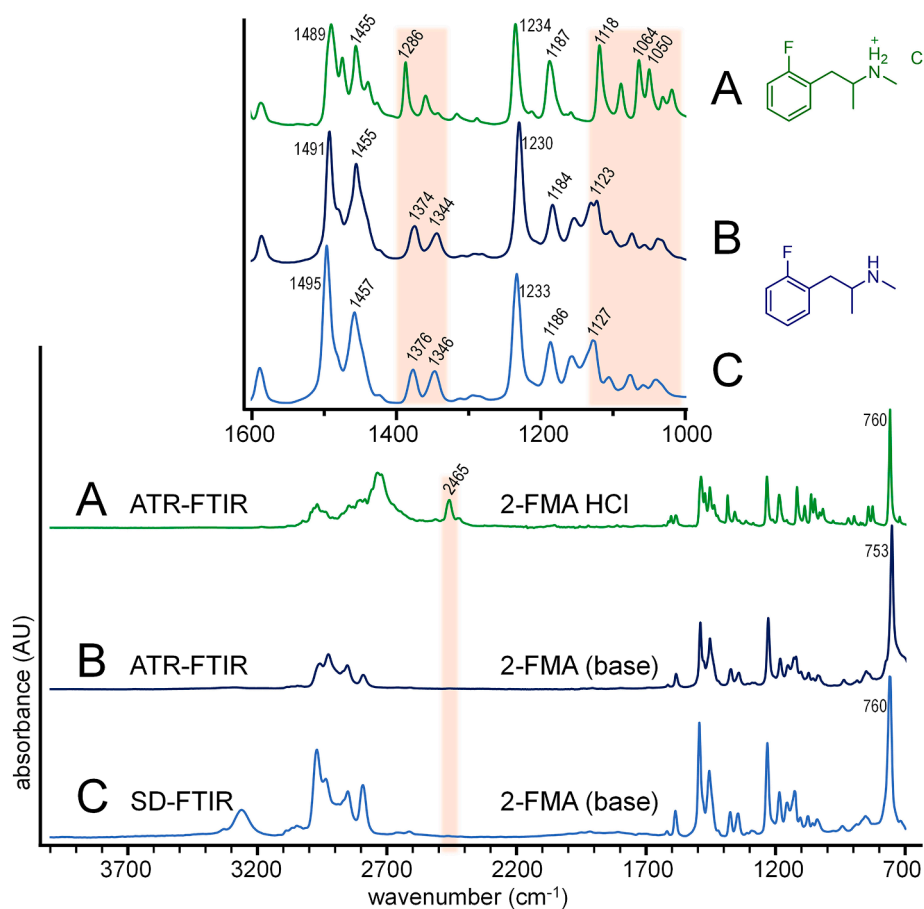

Fig. 9. Comparison of infrared spectra from 2-FMA. Common 2-FMA hydrochloride salt case sample analyzed with ATR-FTIR (A); same sample transferred to its freebase from and subsequently analyzed with ATR-FTIR (B); solid deposition-FTIR spectrum of 2-FMA (C). Top inset zooms in on the spectral fingerprint area. Orange areas mark notable differences between the salt form (A) and the neutral base form (B and C).

encountered NPS to laboratories that do have access to a GC-solid deposition-FTIR system. Although currently unsuitable for identification, obtaining this spectral information may assist in unraveling the identity of unknowns. Due to legislation constraints, sharing a digital spectrum may be far more convenient than sharing and transporting actual seized material. The European RESPONSE project[73], the ADEBAR project[74] and the NPS-datahub[71] are such initiatives to make NPS spectral data available in the public domain. These libraries

are currently still limited in terms of available condensed phase (*viz.* neutral base) FTIR spectra. From the components involved in this study, on-line reference spectra were only available for 3-MEC, dimethylone and pentedrone. These all matched with the spectra observed in our study in terms of most abundant bands in the fingerprint area. However, robust identification requires the creation of a tailor-made GC-solid deposition-FTIR library by analyzing reference standards or case work samples with known active ingredients.

Evasion of narcotic legislation by replacement of substances in new batches

When studying both the unraveled compositions (Table 1) and the product appearances (Fig. 1) it became evident that different batches of similar branding exist. Two different label designs of *Alegria forest fruits* were observed (samples F,G,P,R and U versus samples H,I,L and M). One sample (specimen U) had an additional label with a batch number attached to it. Surprisingly, even the visually similar exhibits showed multiple differences in composition, indicating that the implementation of a new formulation is not always accompanied by a label update. When samples are ranked according to their date of laboratory receival a trend in substance composition (shown in Table 1) becomes apparent that seems to follow changes in the controlled substances legislation in The Netherlands, shown in Table 2. [17] For example, 4-MEC and pentedrone were present in samples received prior to September 2018 but were absent in all samples -even similarly labeled- that were received after that date. Interestingly, these two substances were added to the controlled substances list in April 2018. For *Alegria citrus* samples N, S and Q (Fig. 3, Table 1), three different compositions were identified, of which two compositions contain the currently controlled substances 4-FA, pentedrone and/or ethylone whereas the most recent formulation contains a mixture of four currently still uncontrolled substances. Most strikingly, the labels of these products are identical providing no indication to the users of the changes in the composition.

Since these products are sold openly on the internet as research chemicals, suppliers often state a 'not for human consumption' warning including an ingredient list on their website. For the *Alegria* samples, the composition declared on the supplier's website (situation March 2021) is shown in Table 1. These compositions are completely in-line with actual narcotic legislation in such a way that all mentioned substances are currently unregulated in The Netherlands. Remarkably, the listed ingredients deviate from the composition of the most recent specimen received in the laboratory. Since the laboratory was not able to order advertised products directly from the internet, it could not be tested whether the supplier's statements are incorrect or if a new formulation had become available that had not been encountered by the laboratory yet.

Certain notable particularities were observed: the *Alegria forest fruits* samples H,I,P and R all contained an ~ 0.3 mg/mL peak from the controlled substance 4-FA, although other uncontrolled analogues such as 2-FA, 2-FMA and 4-FMA are also present at levels between 2.4 and 5.2 mg/mL (Fig. 4). Since 4-FA was one of the first NPS with a controlled status in The Netherlands, it does not make any sense for the supplier to add this substance to the formulation. It is therefore hypothesized that this minor contribution originates from either a contamination or an impurity in raw materials without the supplier being aware of this. The same holds for a minor ethylone peak in samples P and R while these samples already contain dimethylone, a substance that seems to be intended as an ethylone replacement following its controlled status since April 2018. It must however be noted that the laboratory does not have any information on the origin of the samples other than the date they were encountered or seized by the police. It is therefore possible that the production date of the samples is well before their date of receival by the laboratory (*e.g.* when an older sample was found and subsequently sent to the laboratory). We therefore cannot and do not intend to state that narcotic legislation was violated. These results do however suggest that suppliers at least follow changes in narcotic legislation and update their formulations accordingly by introducing new, yet uncontrolled NPS in their products.

Conclusion

GC-solid deposition-FTIR is a valuable and efficient technique for NPS identification in multi-component drug formulations such as commercial designer drug mixtures investigated in this study. The highest spectral selectivity in terms of differences in match scores and spectral

Table 2
Recent additions to the controlled substances act (Opiumwet) in The Netherlands.

substance	date added
4-MMC (mephedrone)	09/May/2012
4-FA	25/May/2017
4-MEC	27/Apr/2018
Ethylone	27/Apr/2018
Pentedrone	27/Apr/2018
N-Ethylpentylone (NEP)	17/Nov/2020

bands in the FTIR fingerprint area was observed for ring-positional NPS isomers. For ring isomeric FAs and FMAs true positive match scores below 0.04 were observed in all cases, whereas every false positive match score with another isomer scored above 0.98 on a scale to 1. These substances are very difficult to differentiate with GC–MS, demonstrating the complementary nature of these two techniques. Chromatographic peaks in GC-solid deposition-FTIR were approximately 3 times broader compared to GC–MS, which was attributed to band broadening effects during the deposition process on the cryogenic disk. However, chromatographic resolution was still sufficient for most NPS mixtures. Coelution was only observed in mixtures of correlated isomers. In this situation, several strategies such as rewinding and rescanning of the disk, manual post-processing of peaks and replicate injections while overlaying multiple traces to increase the spectral quality can be applied. An analytical scheme is suggested in which routine GC–MS analysis is followed by GC-solid deposition-FTIR when the GC–MS results reveal the presence of a certain NPS class but are inconclusive with respect to the precise isomeric form.

Combined GC–MS and GC-solid deposition-FTIR results provided insights in timely developments in the composition of commercially available liquids known to contain a mixture of designer drugs. In 12 samples received between 2018 and 2020 that were all labeled *Alegria forest fruits* or *Alegria citrus*, 8 different mixture compositions were identified. Each mixture contained between 4 and 11 different individual NPS. These findings indicate that the producer is frequently updating its recipe, without indicating this on the product label. Comparison of amendments in the local narcotic legislation and established sample compositions revealed a trend of replacement of newly controlled substances by uncontrolled analogues. In earlier batches, ethylone, 4-FA, pentedrone and 4-MEC were found, while later batches contained dimethylone, 2-FA, 4-FMA and an FPM isomer. The former all became controlled substances in 2017 or 2018 in the Netherlands. This indicates deliberate actions to prevent violation of the narcotic legislation by updating formulations with novel, closely related, psychoactive substances.

CRediT authorship contribution statement

Ruben F. Kranenburg: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Visualization, Project administration, Writing - original draft. **Laura I. Stuyver:** Investigation, Writing - review & editing. **Renee de Ridder:** Investigation. **Annique van Beek:** Investigation. **Erik Colmsee:** Investigation. **Arian C. van Asten:** Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.forc.2021.100346.

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