Synthesis, characterisation, detection and quantification of a novel hexyl-substituted synthetic cannabinoid receptor agonist: (*S*)-*N*-(1-amino-3,3-dimethyl-1-oxobutan-2-yl)-1-hexyl-*1H*-indazole-3-carboxamide (ADB-HINACA)

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Highlights

- Novel synthetic cannabinoid ADB-HINACA was detected in a seized herbal sample.
- Synthesis of ADB-HINACA reference standard for comparison.
- Full NMR, ATR-FTIR and GC-EI-MS characterisation of ADB-HINACA is reported.
- Development and validation of GC-EI-MS method of quantification.
- Seized sample was determined by GC-EI-MS to contain 4.9% *w/w* ADB-HINACA.

Abstract

Synthetic cannabinoid receptor agonists (SCRAs) are a continually evolving family of illicit drugs, with novel analogues frequently being detected. This paper reports the detection of a new *N*-hexyl-*1H*-indazole derivative, ADB-HINACA, within a herbal sample seized by law enforcement for the first time in the United Kingdom. The identity of the compound was confirmed *via* the synthesis of a pure ADB-HINACA reference standard and direct spectral comparison by ^{1}H NMR and GC-EI-MS analysis. A full analytical profiling of ADB-HINACA by nuclear magnetic resonance (NMR), attenuated total reflection Fourier-transform infrared (FTIR) spectroscopy and gas chromatography-electron ionisation-mass spectrometry (GC-EI-MS) is reported and shows good concordance between the seized sample and the reference standard. A validated GC-EI-MS method for the routine quantification of the cannabinoid in seized samples (LOD: 1.7 μg/mL, LOQ: 5.8 μg/mL) was also developed and using this method, the seized herbal sample was determined to contain 4.9% *w/w* ADB-HINACA.

Keywords: New psychoactive substances, Synthetic cannabinoid receptor agonists, ADB-HINACA, GC-EI-MS, NMR

1. Introduction

The field of new psychoactive substances (NPS) is constantly evolving, with new compounds entering the illicit drug market annually [1]. Synthetic cannabinoid receptor agonists (SCRAs) are a diverse class of psychoactive substances [2] that exhibit high affinity binding to the cannabinoid CB_1 and CB_2 receptors and mimic the pharmacological effects of the phytocannabinoid, (-)-*trans*-Δ^9-tetrahydrocannabinol (Δ^9-THC) [3-6]. Their usage has been well documented, particularly in the United Kingdom (within rough sleeping/homeless and prison communities) [7-16], and have been responsible for numerous drug-related intoxications resulting in severe clinical effects (aggression, agitation, hallucinations, tachycardia, cardiotoxicity, hepatotoxicity, hyperemesis, respiratory depression, seizures and coma) and/or fatalities globally [3-6, 17-19]. To date, more than 290 SCRAs, traded under names such as *"Clockwork Orange"*, *"Black Mamba"* and *"Spice"* have been detected [1, 20, 21] and are normally encountered as either infused inert plant material (resembling traditional herbal cannabis), paper/card, clothing or dispersed within e-liquids for smoking (either directly or

mixed with tobacco) or vaping [1, 2, 7, 16, 22, 23]. The specific SCRAs identified over time have continually evolved in response to both national and international legislation to both control their manufacture, trafficking, and possession and their availability within producer countries [24-26].

During the last five years, the most prevalent compounds observed on the market, have generally been the valinate and *tert*-leucinate indole- and indazole-3-carboxamide derivatives (*e.g.* 5F-MMB-PICA [27-29], MMB-4en-PICA [30-32], 5F-AMB [27, 33, 34], MMB-4en-PINACA [30, 31, 35], 4F-MDMB-BICA [32, 36, 37], 5F-MDMB-PICA [7, 37-40], MDMB-4en-PICA [7, 30-32, 41, 42], 4F-MDMB-BINACA [7, 32, 43-46] and MDMB-4en-PINACA [7, 30, 47-49]).

However, more recently valinamide and *tert*-leucinamide indole- and indazole-3-carboxamide derivatives (*e.g.* AB-PICA [20, 50], 5F-AB-PINACA [50, 51], AB-CHMINACA [17, 32, 52-60], 5F-ADBICA [50, 60, 61], ADBICA (ADB-PICA) [50, 60, 62], ADB-FUBINACA [50] [60, 63-67], ADB-BUTINACA (ADB-BINACA) [32, 57, 68] and ADB-PINACA [17, 50, 60, 69-71]) have emerged in casework (**Fig. 1**). Huffman *et al.* undertook quantitative structure-activity relationship (QSAR) studies of related synthetic cannabinoids and observed that *N*-alkyl chains containing from 4 to 6 carbon atoms have effective hydrophobic interactions between the synthetic cannabinoid and the binding pockets of the CB_1 and CB_2 receptors [72]. Though the most frequently employed structural modifications have involved replacement of the *N*-alkyl chain on the indole- or indazole core with either a pentyl-, 5F-pentyl-, *para*-fluorobenzyl-, pent-4-enyl- or methylcyclohexyl- moieties (**Fig. 1**) to-date no *N*-hexyl-*1H*-indole or indazole derivatives have been encountered and as such their pharmacology, pharmacokinetics and/or toxicological profiles have not been fully elucidated. Therefore, there is an increased need for new reliable methods of their detection and to reduce potential drug-related harms should this subclass become more prominent on the market.

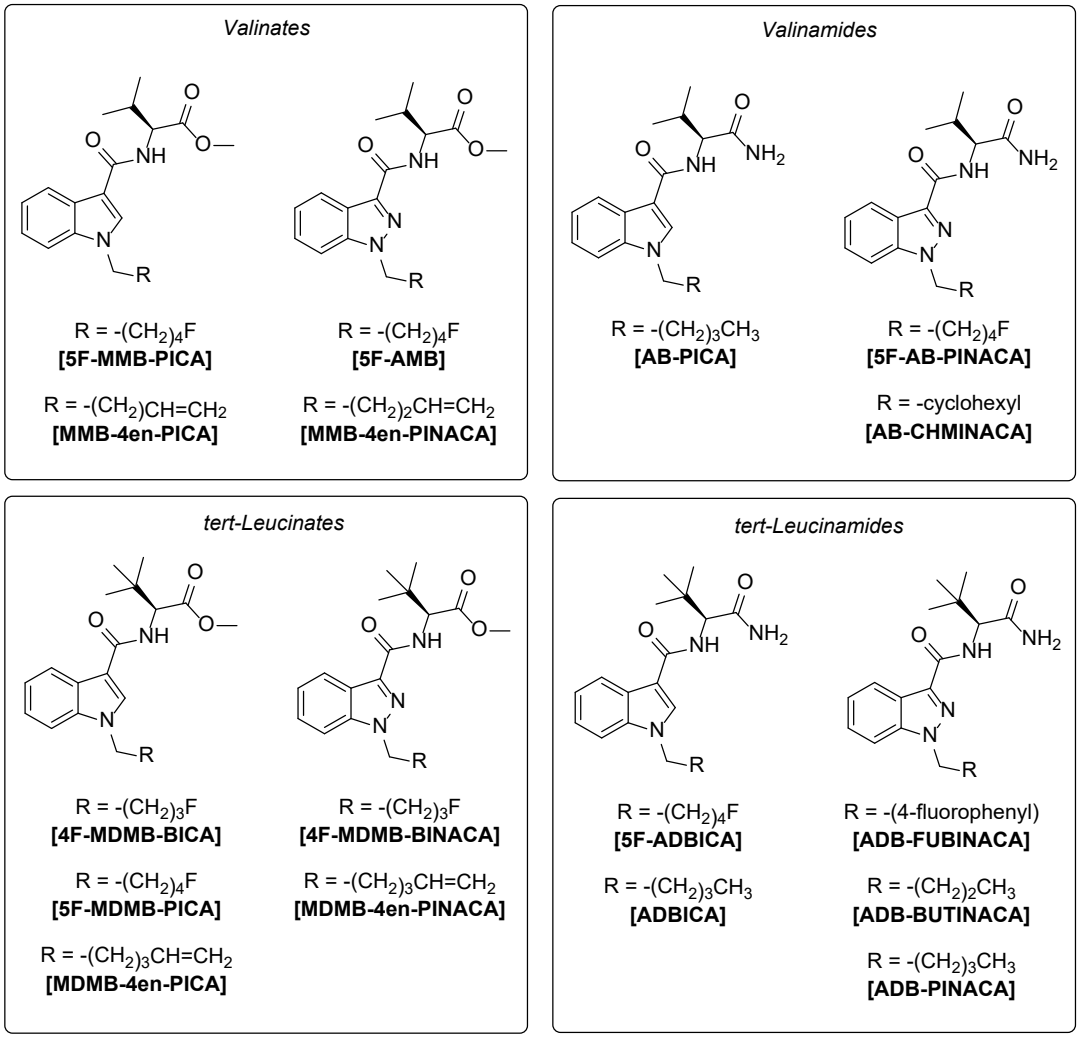
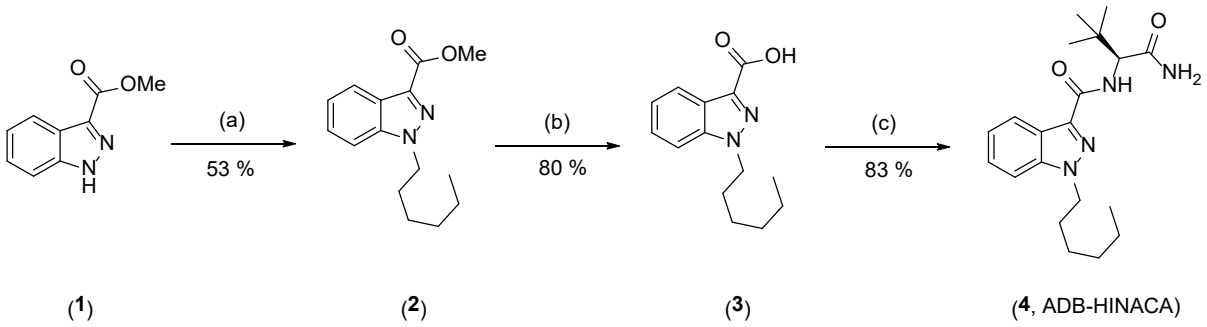


Fig. 1. Common indole- and indazole-3-carboxamide-based synthetic cannabinoid receptor agonists.

This paper presents the synthesis, full structural characterization and development of a validated gas chromatography-electron ionisation-mass spectrometry (GC-EI-MS) approach for quantification of (*S*)-*N*-(1-amino-3,3-dimethyl-1-oxobutan-2-yl)-1-hexyl-*1H*-indazole-3-carboxamide (**4**, ADB-HINACA; ADB-HEXINACA, **Sch. 1**), a novel *N*-hexyl substituted synthetic cannabinoid analogue of ADB-BUTINACA, found in herbal material (GM48, **Fig. 2**) obtained in Ashton-under-Lyne, Tameside, UK (23rd March 2021). Analytical features of ADB-HINACA were characterized by ^{1}H- and ^{13}C{^{1}H}- nuclear magnetic resonance (NMR) spectroscopy, GC-EI-MS and attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy.



Sch. 1. Synthesis of ADB-HINACA (**4**). *Reagents & Conditions:* (a) 1-bromohexane, NaH, DMF, 0 ºC to r.t., 48 h; (b) NaOH (1 M aq.), MeOH, reflux, 48 h; (c) (*S*)-2-amino-3,3-dimethylbutanamide.HCl, EDC.HCl, HOBt, NEt_3, DMF, r.t., 24 h.

Fig. 2. Photograph of suspected *"Spice"* impregnated vegetable matter (GM48, 52.8 mg) obtained in Ashton-under-Lyne, Tameside, UK (23rd March 2021).

Though NPS Discovery has recently confirmed ADB-HINACA (referred to as ADB-HEXINACA), in a sample seized in Florida, USA (12th April 2021), their disclosed analytical data (GC-EI-MS and LC-qTOF-MS only) for this novel cannabinoid is limited [73] and there has been no comprehensive analytical profiling or development of validated chromatographic methods for this substance. To the best of our knowledge, this is the first paper detailing the synthesis, comprehensive structural characterization of ADB-HINACA and provision of a validated GC-EI-MS method for the routine quantification of the SCRA within bulk samples, which will be valuable as a reference point for future forensic analysis of this and related compounds.

2. Materials and methods

The reference standard of (*S*)-*N*-(1-amino-3,3-dimethyl-1-oxobutan-2-yl)-1-hexyl-*1H*-indazole-3-carboxamide (**4**, ADB-HINACA; ADB-HEXINACA) was synthesized, purified and obtained as a stable, colourless powder (35% overall yield) using an adaptation of the protocols reported by Cannaert *et al.* [32] (see Supplementary Information for synthetic procedures and characterization data).

^{1}H NMR and ^{13}C{^{1}H} NMR spectra (10.0 mg/1.0 mL in $CDCl_3$) were acquired on a JEOL JMN-ECS-400 (JEOL, Tokyo, Japan) NMR spectrometer operating at a proton resonance frequency of 400 MHz, referenced to the residual solvent peak ($CDCl_3$: ^{1}H NMR δ = 7.26 ppm, ^{13}C{^{1}H} NMR δ = 77.16 ppm, respectively) [74]. Infrared spectra were obtained in the range 4000 – 400 cm^{-1} using a Thermo Scientific Nicolet iS10ATR-FTIR instrument (Thermo Scientific, Rochester, USA).

The suspected *"Spice"* sample (GM48, 52.8 mg, **Fig. 2**) was obtained in Ashton-under-Lyne, Tameside, UK and provided, to MANchester DRug Analysis and Knowledge Exchange (MANDRAKE), by Greater Manchester Police on 23rd March 2021 in accordance with Manchester Metropolitan University's Home Office license requirements and agreed procedures.

2.1 Gas chromatography–electron ionisation-mass spectrometry (GC–EI-MS)

GC-EI-MS analysis was performed using an Agilent 7890B GC and a MS5977B mass selective detector (Agilent Technologies, Wokingham, UK). The mass spectrometer was operated in the electron ionisation mode at 70 eV. Separation was achieved with a capillary column (HP-5MS, 30 m length, 0.25 mm i.d., 0.25 μm film thickness) with helium as the carrier gas at a constant flow rate of 1.2 mL/min. The following oven temperature program was used: 50 to 290 °C at 30 °C/min, hold for 4 min for a 12 min total runtime. A 2 μL injection volume was used, with a split ratio of 50:1. The injector and the GC interface temperatures were both maintained at 280 °C, respectively. The MS source and quadrupole temperatures were set at 230 °C and 150 °C. Mass spectra were obtained in full scan mode (*m/z* = 40–550) for ADB-HINACA (**4**) (t_R = 10.83 min) and eicosane (t_R = 7.20 min), respectively.

Method validation: The GC-EI-MS method was validated, in accordance with the ICH guidelines [75], using the following parameters: linearity, precision, accuracy, limit of

detection (LOD) and limit of quantification (LOQ). *Linearity and precision:* six replicate injections of the calibration standards were achieved under identical conditions. The %RSD was calculated for each replicate test sample and the linearity (r^2) of the calibration was determined. *Accuracy (percentage recovery study):* determined from spiked samples prepared in triplicate at three levels over a range of 80 – 120% of the target concentration (150 µg/mL). The percentage recovery and %RSD were calculated for each of the replicate samples. *Limits of detection and quantification:* six replicate injections of the calibration standard (50 µg/mL) were performed. The limits of detection and quantification were determined based on a signal-to-noise ratio of 3:1 and 10:1, respectively [75]. Signal-to-noise ratios were measured using the auto-root-mean-squared (Auto-RMS) algorithm from the Agilent MassHunter Qualitative Analysis software. *Calibration standards:* 10.0 mg of ADB-HINACA (**4**) was weighed accurately into 10.0 mL clear glass class A volumetric flask and diluted to volume with methanol to give a solution containing (**4**) at 1.0 mg/mL. This solution was then further diluted with methanol and eicosane (100 µg/mL in methanol) added (in each case) to give calibration standards containing 50.0 µg/mL, 100.0 µg/mL, 150.0 µg/mL, 200.0 µg/mL and 250.0 µg/mL of (**4**) and the internal standard at 10.0 µg/mL. The five standards were injected six times. *Test sample:* 52.8 mg of the homogenised sample (GM48) was suspended in 1.0 mL methanol, sonicated for 5 min and filtered through a 0.45 µm PTFE syringe filter. For initial qualitative analysis, a 300 µL aliquot of the resulting solution was diluted with 700 µL methanol and injected. For quantitative analysis, a 200 µL aliquot of this solution was further diluted with 700 µL methanol and 100 µL eicosane (100 µg/mL in methanol; 10 µg/mL final internal standard concentration).

3. Results and Discussion

3.1 Nuclear Magnetic Resonance (NMR) Spectroscopy

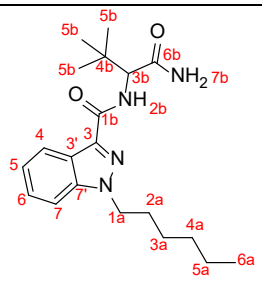
The reference standard of (*S*)-*N*-(1-amino-3,3-dimethyl-1-oxobutan-2-yl)-1-hexyl-*1H*-indazole-3-carboxamide (**4**) was synthesized, purified and obtained as a stable, colourless powder (**Sch. 1**, 35% overall yield) using an adaptation of the protocols reported by Cannaert *et al.* [32] The purity (>99.5%) of (**4**) was calculated by ^{1}H-NMR using the relative concentration determination method described by Pauli *et al.* [75] Retention of enantiomeric fidelity [(*S*)-enantiomer] within the final product is assumed through both the use of commercially available, enantiopure (*S*)-2-amino-3,3-dimethylbutanamide hydrochloride

(≥99% *e.e.*) and that racemization is mechanistically unlikely due to the achirality of the 1-hexyl-*1H*-indazole-3-carboxylic acid precursor (**3**) [31, 57]. The synthesised ADB-HINACA (**4**) standard was analysed by NMR to facilitate its characterisation. ^{1}H and ^{13}C{^{1}H} NMR spectra of compound (**4**) are shown in **Fig. 3a** and **Fig. 4**, respectively. The full assignment of ^{1}H and ^{13}C{^{1}H} NMR signals of ADB-HINACA (**4**) is presented in **Table 1**. The 2D correlation NMR spectra are available within the Electronic Supplementary Information (**Fig. S9** – **S12**). The assignment relied on correlation spectroscopy (COSY; **Fig. S9**) to observe ^{1}H-^{1}H couplings, heteronuclear multiple quantum coherence (HMQC; **Fig. S10**) for one-bond ^{1}H-^{13}C couplings, heteronuclear multiple bond correlation (HMBC; **Fig. S11**) for two- or three-bond ^{1}H-^{13}C couplings (acquired at a $^{2}J_{HC}$ = 8 Hz), and ^{1}H-^{1}H nuclear Overhauser effect spectroscopy (NOESY; **Fig. S12**) to determine the spatial proximity of protons.

In the ^{1}H NMR spectrum (**Fig. 3a**), the triplet at 0.92 – 0.84 ppm (3H) can be assigned to the H6a methyl at the end of the *N*-hexyl chain. In the COSY spectrum, this signal couples to a multiplet at 1.41 – 1.25 (6H), which is an overlap of H5a, H4a and H3a. One of these, in turn, couples to H2a [1.94 ppm (p, 2H)], which couples to H1a [4.39 ppm (t, 2H)]. The latter is the most deshielded of the aliphatic chain due to its proximity to the indazole nitrogen.

C1a, C2a and C6a can then be easily assigned *via* HMQC analysis, but C3a, C4a and C5a require HMBC analysis due to the overlap of their corresponding proton signals. In the HMBC spectrum, only two signals in the aliphatic region exhibit a long-distance coupling to H1a: one at 29.83 ppm (C2a) and the other at 26.59 ppm. The latter can thus be assigned to C3a due to its relative proximity to H1a (3 bonds away). As for the remaining aliphatic signals, they can be assigned based on chemical shift: deshielding can be observed from C6a (14.13 ppm), to C5a (22.64 ppm) and C4a (31.43 ppm), due to an increasing inductive effect along the carbon chain. The chemical shift of C3a (26.59 ppm) was lower than that of C4a (31.43 ppm) due to the effect of the nitrogen substituent in its γ position [76].

Table 1. ^{1}H NMR and $^{13}C\{^{1}H\}$ NMR data for (**4**) (ADB-HINACA) reference standard (in $CDCl_3$).



(**4**, ADB-HINACA)

Assignment	^{1}H (ppm)	$^{13}C\{^{1}H\}$ (ppm)
3	-	140.94
3’	-	122.97
4	8.34-8.29 (m, 1H)	122.60
5	7.32 – 7.16 (m, 1H)	122.72
6	7.46 – 7.36 (m, 2H)	126.70
7	7.46 – 7.36 (m, 2H)	109.51
7’	-	136.53
1a	4.39 (t, J = 7.3 Hz, 2H)	49.66
2a	1.94 (p, J = 7.2 Hz, 2H)	29.83
3a	1.41 – 1.25 (m, 6H)	26.59
4a	1.41 – 1.25 (m, 6H)	31.43
5a	1.41 – 1.25 (m, 6H)	22.64
6a	0.92 – 0.84 (t, J = 7.0 Hz, 3H)	14.13
1b	-	162.87
2b	7.67 (d, J = 9.6 Hz, 1H)	-
3b	4.54 (d, J = 9.5 Hz, 1H)	59.73
4b	-	34.77
5b	1.15 (s, 9H)	26.89
6b	-	172.97
7b	5.94 (s, 1H), 5.46 (s, 1H)	-

In the ^{1}H-^{1}H NOESY spectrum, H1a couples to an aromatic multiplet at 7.46 – 7.36 (2H), which is likely an overlap of H7 (due to its proximity) and another aromatic proton. COSY also shows coupling between the proton signal at 8.34-8.29 (m, 1H) and that at 7.32 – 7.16 (m, 1H), but not between the former and 7.46 – 7.36. This means that the multiplet at 8.34-8.29 ppm can be assigned to H4, the multiplet at 7.32 – 7.16 to H5, and the overlapped 7.46 – 7.36 ppm signal

to H6 and H7. The respective aromatic carbon signals can be attributed by HMQC. In HMBC, H4 couples to a quaternary carbon signal associated with C3 (140.94 ppm).

The ^{1}H singlet at 1.15 ppm (9H) can be attributed to the *tert*-butyl protons H5b but also to a quaternary ^{13}C signal at 34.77 ppm (C4b) and another signal at 59.73 ppm (C3b). The latter corresponds to a ^{1}H doublet at 4.54 ppm (1H) which can be assigned to H3b. According to the COSY spectrum, H3b couples (J = 9.6 Hz) to a doublet at 7.67 ppm (1H) which can be assigned to amide proton H2b. The primary amide protons H7b can be attributed to a pair of broad singlets at 5.94 (1H) and 5.46 (1H) ppm. Both couple only to each other in COSY. NOESY analysis confirms the proximity between H7b, H3b and H5b. Carbonyl signals C1b (162.87 ppm) and C6b (172.97 ppm) were assigned based on their chemical shifts.

With this assignment completed, the seized sample GM48 underwent ^{1}H NMR analysis (**Fig. 3b**) and the resulting spectrum was compared to that of the ADB-HINACA (**4**) reference standard (**Fig. 3a**). All the signals observed in the reference standard spectrum were concordant with those in the seized sample spectrum, indicating that GM48 contained ADB-HINACA. The ^{1}H-NMR spectrum of GM48 also showed additional peaks at 8.14, 5.36 and 1.86-1.46 ppm, indicating the presence of unknown impurities, potentially phytochemicals, extracted from the base plant material.

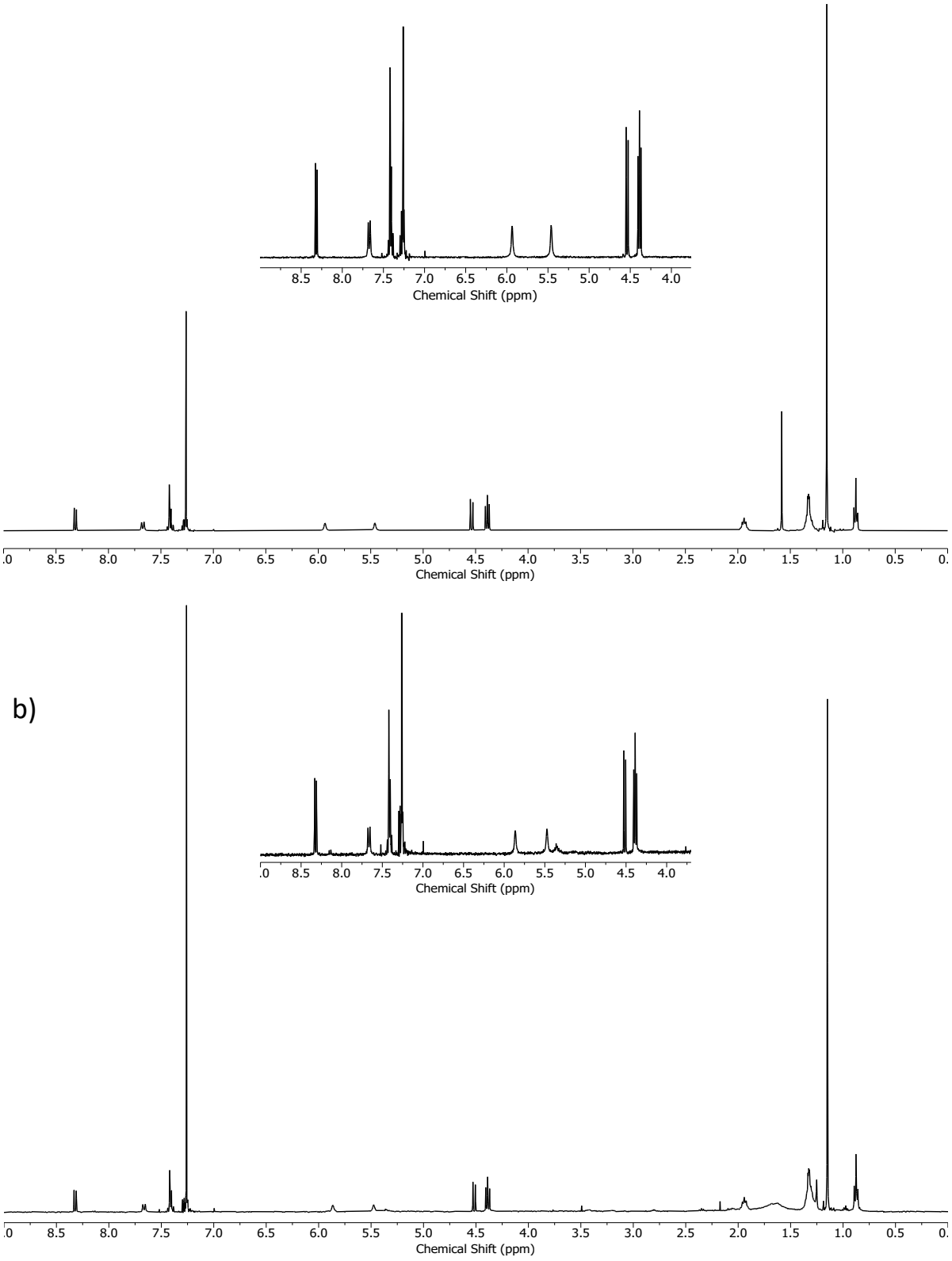


Fig. 3. (a) ^{1}H NMR (400 MHz, 10.0 mg/1.0 mL $CDCl_3$) spectrum of synthesised ADB-HINACA (**4**) reference standard; (b) ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of seized suspected *"Spice"* sample (GM48). *Note:* * = residual water.

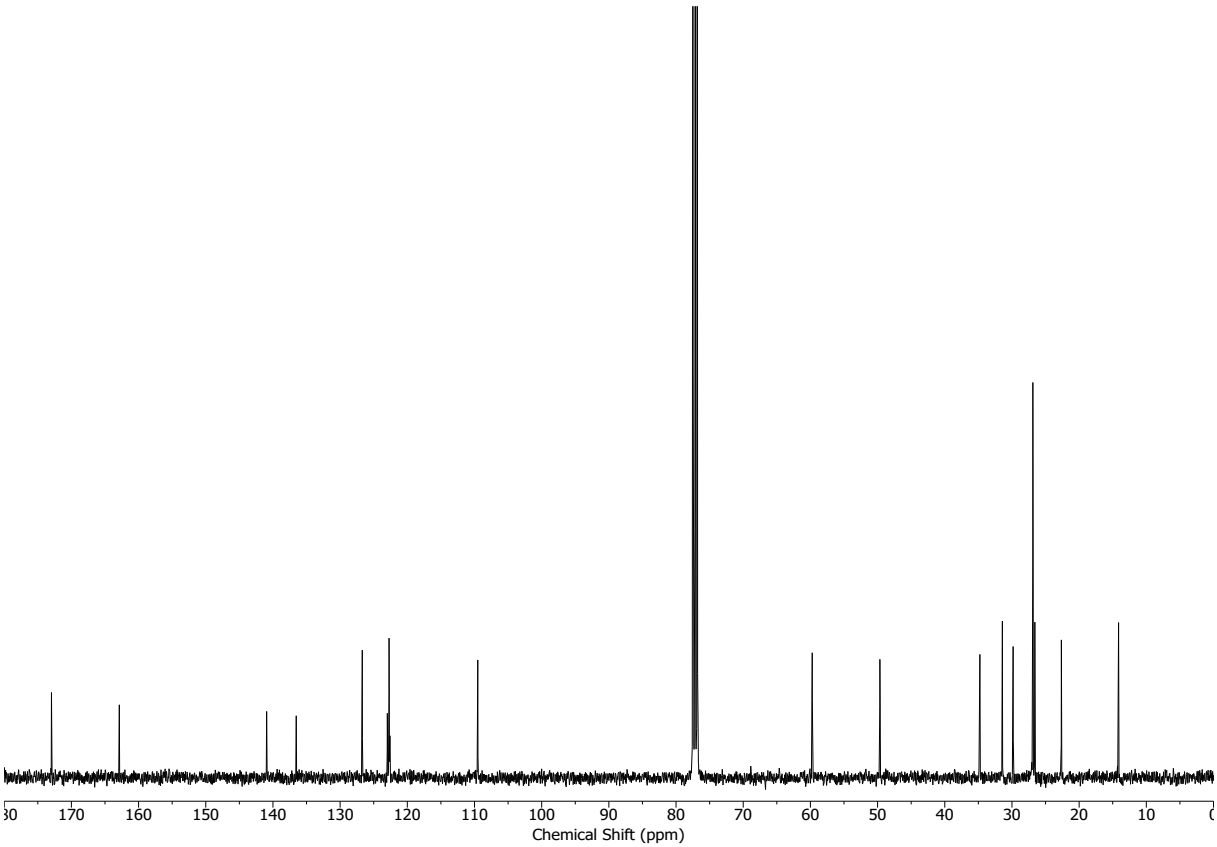


Fig. 4. $^{13}C\{^{1}H\}$ NMR (100 MHz, 10.0 mg/1.0 mL $CDCl_3$) spectrum of synthesised ADB-HINACA (**4**) reference standard.

3.2 Attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy

The infrared spectrum (see Electronic Supplementary Information, **Fig. S13**) of (**4**) was collected on an ATR–FTIR spectrometer and shows a strong amide carbonyl absorption band at 1651 cm^{-1} as well as broad bands between 3500-3000 cm^{-1}, characteristic of the N–H stretching within the amide groups. The spectrum also exhibits weak aromatic stretching bands around 3060 cm^{-1}, indicative of an aromatic nucleus, and alkyl C–H stretching vibrations in the region 2956 – 2860 cm^{-1}. Normally the fingerprint region (500 – 1600 cm^{-1}) is complex and it can be difficult to differentiate vibrational bands, however, comparison with the data reported by Dybowski *et al.* [49] allows the tentative assignment of structural motifs such as the indazole ring (1366 cm^{-1}) and the *tert*-butyl moiety (1212 cm^{-1}) within the spectral region.

3.3 Gas chromatography-electron ionisation-mass spectrometry (GC-EI-MS)

The qualitative GC-EI-MS method (*ca.* 12 min) used required an extremely straightforward solvation of the samples in methanol (250.0 μg/mL containing 10.0 μg/mL eicosane as internal standard) followed by direct injection into the instrument. No derivatization step was required. An exemplar total ion chromatogram is presented in **Fig 5a**. The use of GC-EI-MS facilitated the visualization of the mass spectral data for (**4**), and this is presented in **Fig 5b**. The GC-EI-MS total ion chromatogram of a methanolic extract (containing 10.0 μg/mL eicosane as internal standard) of the seized material (GM48) and the corresponding electron ionisation (EI) mass spectrum are presented in **Fig. 5c** and **5d**, respectively. The data indicates that the seized sample contains a single component (t_R = 10.83 min) and the EI spectrum is in good agreement with both the synthesised reference standard of (**4**) (**Fig. 5b**) and the EI-MS information reported by NPS Discovery [73].

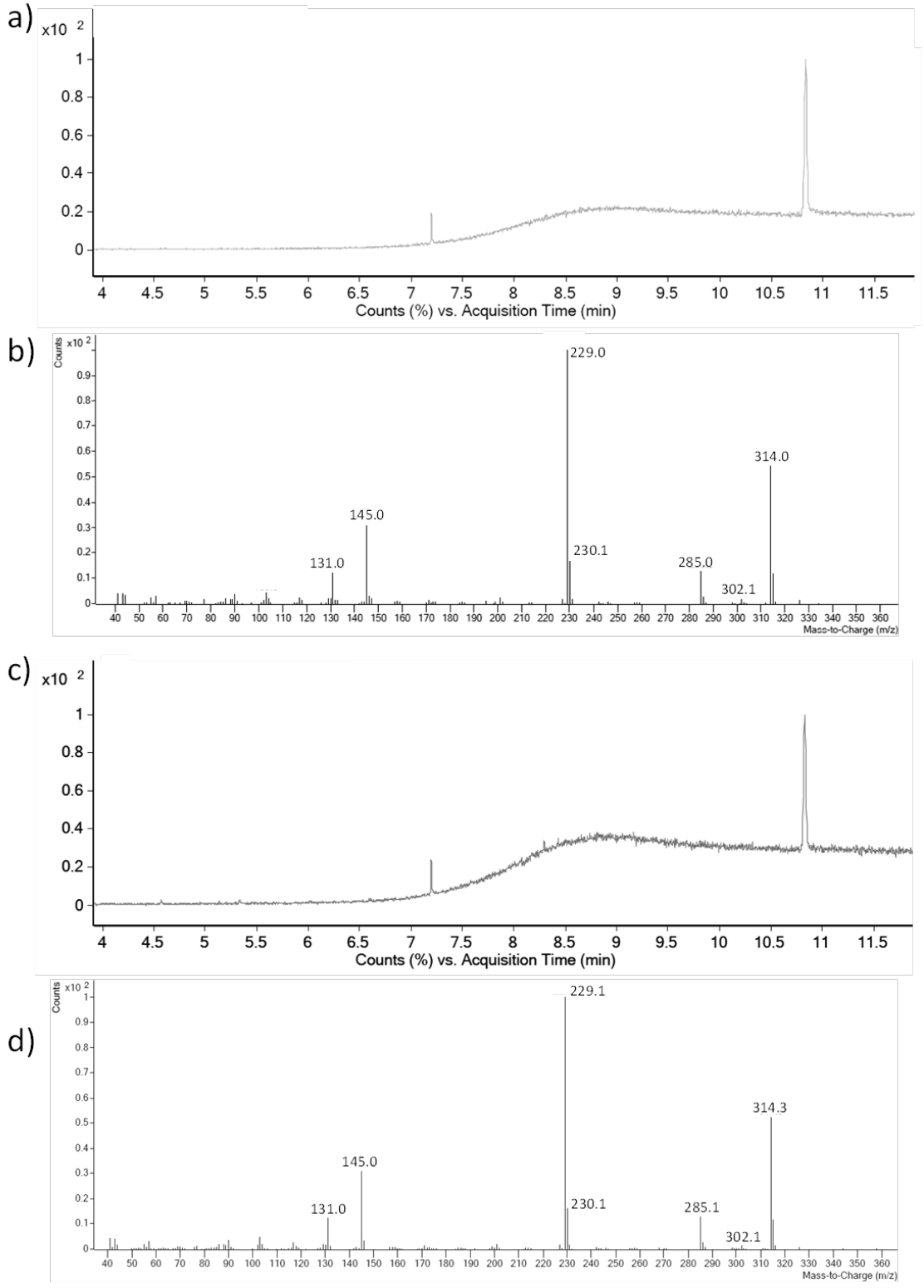
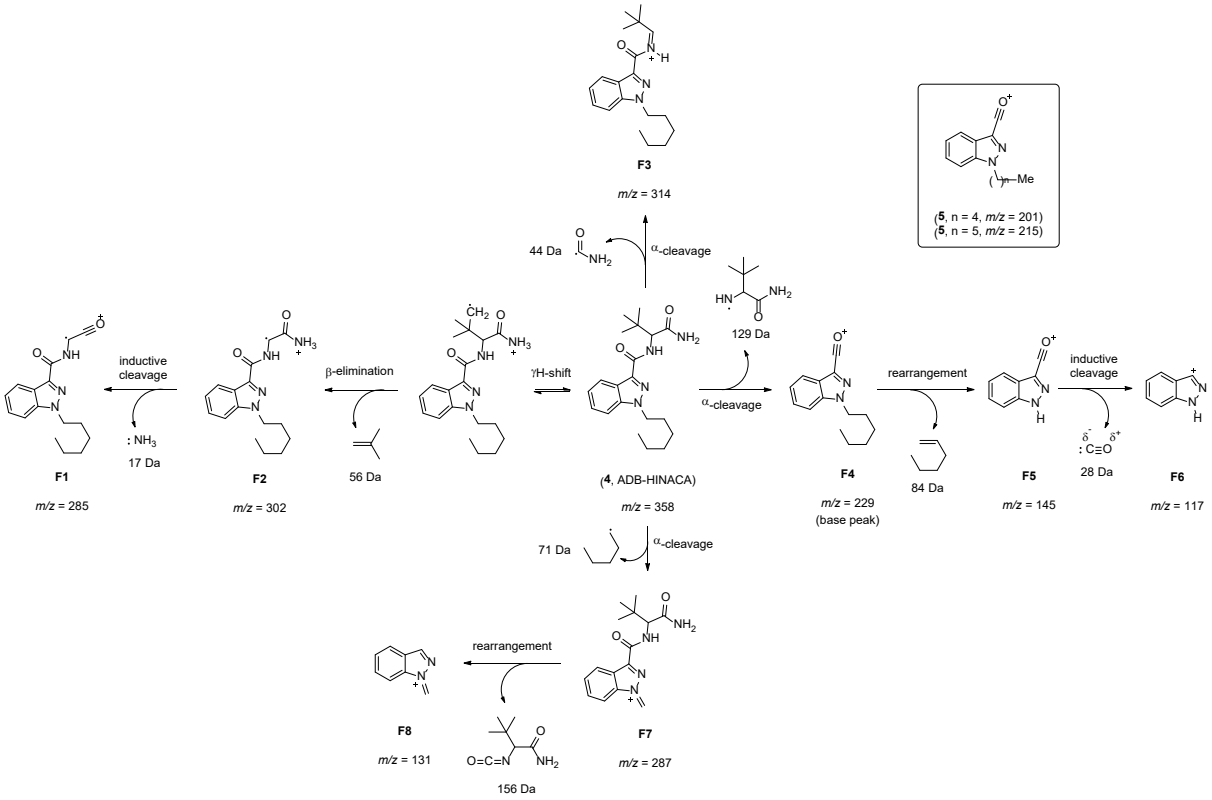


Fig. 5. (a) Total ion chromatogram of synthesised (**4**) reference standard; (b) EI-MS spectrum (+ve ion mode) of (**4**) reference standard (t_R = 10.8 min); (c) Total ion chromatogram of seized suspected *"Spice"* sample (GM48); (d) EI-MS spectrum (+ve ion mode) of peak (t_R = 10.83 min) in seized suspected *"Spice"* sample (GM48). *Note:* t_R (eicosane) = 7.20 min. See Section 2.2 for experimental details.

The structures of the molecular ion (*m/z* = 358) and diagnostic fragment ions are presented in **Sch. 2**. The proposed fragmentation pathway for (**4**) is similar to those reported for similar synthetic cannabinoids of the carboxamide class [49]. The acylium-indazole-hexyl ion (**F4**) at *m/z* = 229 is the most intense ion (base peak) in the spectrum and is produced *via* α-cleavage of the *tert*-leucinamide residue attached to the carboxamide group. The structure of **F4** is similar to the structure of the base peaks occurring in the EI-MS spectrum of other synthetic cannabinoids containing a carboxyindazole core [49, 77]. Examination of the corresponding ions *i.e.* acylium-indazole-butyl (**5**, *n* = 4, *m/z* = 201) [78, 79] and acylium-indazole-pentyl (**5**, *n* = 5, *m/z* = 215) [77] within the homologous series ADB-BUTINACA → ADB-PINACA → ADB-HINACA strongly implies the presence of a *N*-substituted-hexyl sidechain. Acylium-indazole ion **F5** (*m/z* = 145) further confirms the presence of indazole-carboxy moiety in the examined molecule and arises *via* rearrangement and subsequent loss of 1-hexene (84 Da). The acylium-indazole ion undergoes further inductive cleavage of carbon monoxide (28 Da), under electron-ionization conditions, to give **F6** (*m/z* = 117). The iminium ion (**F3**) at *m/z* = 314 in **Fig. 5b** is proposed *via* an alternative α-cleavage of the *tert*-leucinamide residue with the charge initiation on the internal amide moiety. Formation of the methylidene-indazolium ion **F8** (*m/z* = 131), originates through α-cleavage of the *N*-alkyl chain starting with charge localization on the indazole core. The intermediate fragment ion **F7** (*m/z* = 287) is not overly abundant, but is observed in both the EI spectrum (**Fig. 5b**) and within the mass spectral data reported by NPS Discovery [73]. A subsequent acyclic internal rearrangement from the *tert*-leucinamide moiety leads to the formation of the methylidene-indazolium ion **F8** (*m/z* = 131). The ion **F2** (*m/z* = 302) is suggested to be a product of a McLafferty rearrangement initiated by γ-hydrogen shift to the nitrogen of the terminal amide, leading to a charge localization and subsequent loss of isobutylene (56 Da) from the molecular ion [77]. The formation of the minor secondary ion **F1** (*m/z* = 285) is advocated through inductive cleavage and loss of neutral ammonia (17 Da) from **F2** and has been observed in structurally related cannabinoids [77].



Sch. 2. Proposed electron ionisation (EI) fragmentation pathway(s) for (**4**).

The quantitative GC-EI-MS method was developed and validated in accordance with the ICH guidelines [75]. Results of the method validation are reported in **Table 2**. Calibration standards were prepared and demonstrated a linear response ($r^2 = 0.997$) over a 50.0 – 250.0 µg/mL range with satisfactory repeatability (RSD = 3.2-5.2%, n = 6). The limits of detection and quantification for ADB-HINACA were determined, based on the signal to noise (S/N) ratio, as being 1.7 and 5.8 µg/mL, respectively. The accuracy (percentage recovery study) of the method was determined using a percentage recovery study (**Table 3**). Spiked samples were prepared in triplicate at three concentration levels over a range of 80–120% of a target concentration (150 µg/mL). Experimental concentration is determined using the developed calibration and compared with the theoretical concentration (assay recovery). The relative error shows how the mean assay recovery diverges from an expected 100%. Acceptable recoveries (100.0±0.4%) were obtained at all three measured concentrations.

The GC-EI-MS approach was deemed suitable for the analysis of the street sample (GM48). The sample was reanalysed (in triplicate) and quantification of ADB-HINACA (**4**) was performed in full scan mode (m/z = 40–550). The quantitative results confirmed that the diluted sample contained (**4**) at a concentration of 156.5 µg/mL, which correspond to 4.9±0.1% *w/w* in the original plant sample (based on total sample weight). This equates to 2.61±0.06 mg within

the 52.6 mg bulk sample. As a comparison, recent seizures of 5F-MDMB-PINACA and AMB-FUBINACA (n = 16) within Greater Manchester were found to contain < 0.10 – 9.15% *w/w* and 1.55 – 5.85% *w/w*, respectively [57]. It should be noted that this recent result may not truly reflect the typical concentrations of ADB-HINACA samples nationally, due to the small sample size.

Conclusion

This study reports the first synthesis, comprehensive analytical profiling (^{1}H, ^{13}C{^{1}H} NMR, ATR-FTIR and GC-EI-MS) of the novel synthetic cannabinoid: (*S*)-*N*-(1-amino-3,3-dimethyl-1-oxobutan-2-yl)-1-hexyl-*1H*-indazole-3-carboxamide (**4**, ADB-HINACA) confirmed within a seized bulk sample. In addition to the synthetic methods and spectral data the paper presents the development of a rapid, validated GC-EI-MS method for the routine detection (within 12 mins) and quantitative analysis (LOD: 1.7 μg/mL, LOQ: 5.8 μg/mL, respectively) for (**4**) which is suitable for processing of bulk samples encountered in casework. The seized sample was confirmed to contain ADB-HINACA at a concentration of 4.9±0.1% *w/w* (equating to 2.61±0.06 mg within the bulk sample) which is similar to previously reported concentrations within the Greater Manchester region. It is envisaged that the data presented herein will be valuable as a reference point for future analysis of this novel cannabinoid and structurally related compounds as they emerge on the illicit drug market.

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Declaration of interests

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.

Table 2. GC-EI-MS validation data for the quantification of ADB-HINACA (**4**). See **Fig. 5a** for representative total ion chromatogram.

Analyte	t_R (min)	A_s	N (plates)	H (x10^{-5} m)	r^2	LOQ[b] (µg/mL)	LOD[c] (µg/mL)	Precision (%RSD, n = 6)				
								50 µg/mL	100 µg/mL	150 µg/mL	200 µg/mL	250 µg/mL
Eicosane (IS)	7.20	0.97	3360499	0.89	–	–	–	–	–	–	–	–
4	10.83	1.03	1070545	2.80	0.997[a]	5.8	1.7	5.2	4.2	4.0	3.2	3.7

Key: [a] y = 0.0347x + 2.366; [b] Limit of detection (determined using a signal-to-noise ratio of 3:1); [c] Limit of quantification (determined using a signal-to-noise ratio of 10:1).

Table 3. GC-EI-MS percentage recovery of ADB-HINACA (**4**) determined from three replicate concentrations near the target test concentration of 150 µg/mL (80%, 100% and 120%). See Section 2.2 for experimental details.

Analyte	Assay recovery			Average recovery (%)	%RSD (*n* = 3)	Relative Error[a] (%)
	120 µg/mL (%, *n* = 3)	150 µg/mL (%, *n* = 3)	180 µg/mL (%, *n* = 3)			
4	100.0	101.8	99.4	100.4	1.3	0.4

Key: [a]Deviation between the average experimental recovery and a 100% recovery.

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