Forensic Science International 292 (2018) 115–124

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Chemical profiling of the street cocktail drug 'nyaope' in South Africa using GC–MS I: Stability studies of components of 'nyaope' in organic solvents

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ARTICLE INFO

Article history:
Received 27 March 2018
Received in revised form 30 July 2018
Accepted 1 August 2018
Available online 9 August 2018

Keywords:
Cannabis
Heroin
Nyaope
Gas chromatography–mass spectrometry

ABSTRACT

Nyaope, a street drug commonly found in South Africa, is a mixture of low grade heroin, cannabis products, antiretroviral drugs and other materials added as cutting agents. It is a highly physiologically addictive substance which is smoked by users. Little work has been published on the chemical analysis and profiling of nyaope. Sample preparation prior to chromatographic or spectrometric analysis normally involves dissolution of the sample in an organic solvent. This study determined the most suitable organic solvent in which the common components of nyaope, namely Δ^9-tetrahydrocannabinol, diamorphine, caffeine, dextromethorphan, phenacetin and the antiretrovirals efavirenz and nevirapine, which have different chemical characteristics, are stable during extraction and prior to analysis of nyaope samples i.e. autosampler stability. Street samples of cannabis (Δ^9-tetrahydrocannabinol), heroin (diamorphine) and antiretrovirals were mixed to mimic a nyaope sample and dissolved in the organic solvents dichloromethane, ethanol, ethyl acetate, hexane, isopropanol and tertiary butyl alcohol. Analysis was performed after intervals of 0, 1, 6, 8, 24, 48 and 72 h, prior to analysis by gas chromatography–mass spectrometry. Tertiary butyl alcohol resulted in the most stable extracts of the main nyaope components after 72 h of storage. The analysis was also repeated on actual street samples of nyaope. These results show that tertiary butyl alcohol is a suitable solvent for sample preparation for the identification, comparison and profiling of nyaope samples.

1. Introduction

The abuse of illicit drugs such as cocaine, methamphetamine and heroin is a problem that is prevalent in many urban areas in countries around the world including South Africa. The clamp-down on the abuse of illicit drugs through legislation has led to existing illicit drugs being reintroduced with new names to disguise them from the authorities. A potent cocktail mixture of low grade heroin smoked with cannabis has been introduced in South Africa as 'nyaope'. The abuse of the cocktail drug nyaope, in South Africa, has increased in recent years mainly amongst young African and Coloured males [1,2]. This often results in these young males turning to theft, losing their jobs and/or dropping out of school. The nyaope users, who are mostly from poor backgrounds, often resort to criminal activities to sustain their drug habit which includes stealing anything valuable that they can lay their hands on [2–4]. Pregnant woman are not excluded from the abuse of nyaope, which has detrimental consequences to the unborn babies [5].

An understanding of the relative proportions of the illicit drug components and chemical profiling of nyaope, which mainly contains diamorphine in combination with cannabis and/or other psychotropic substances [6,7], will assist relevant stakeholders in prosecuting those involved in the manufacture, trafficking and distribution of the drug.

Chemical analysis frequently involves dissolution of a drug prior to using an instrumental method. A solvent in which the components of nyaope are soluble and stable is necessary for the profiling of impurities since chemical degradation of the sample and/or artefact formation may result in erroneous chemical profiles of the nyaope samples. A similar principle has been applied to amphetamine profiling [8] and to piperazines [9].

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https://doi.org/10.1016/j.forsciint.2018.08.001

Finding a suitable solvent for nyaope is particularly problematic due to the different chemical classes of drugs likely to be encountered.

The use of antiviral drugs (ARVs) as cutting agents for nyaope has reportedly led to health professionals being robbed or even corrupt officials selling the ARVs [10–12]. HIV positive patients are also either robbed or sell the ARVs themselves thereby defaulting on their treatment [10–12]. The abuse of nyaope mixtures containing ARVs by pregnant women results in the development of ARV pre-treatment resistance associated with abnormal intrauterine growth, neonatal abstinence syndrome as well as abnormal behaviour for the unborn child [5]. Some of the common psychiatric side-effects of antiretroviral drugs include agitation, anxiety, hallucinations, insomnia, lethargy, nervousness, mood disorders, depression, suicidality, antisocial behaviour, psychosis, catatonia, delirium and vivid dreams [13]. It is the hallucinatory nature of the antiretrovirals which cause them to be included in the street drug. Heroin is an addictive drug and the presence of ARVs makes nyaope an even more addictive drug mixture which can cause violent stomach cramps [14].

ARVs are high molecular weight compounds which makes their analysis using GC–MS difficult. Only two ARVs, efavirenz (EFV) and nevirapine (NVP) have successfully been analysed using GC–MS [15,16]. EFV is reported to be a hallucinogen [17] while no psychoactive effects have been reported for nevirapine [13]. A combination of the two hallucinogens EFV and Δ^9-tetrahydrocannabinol may result in stronger hallucinating power of nyaope.

Both EFV and NVP are shown to be stable in methanol when stored for 24 h at ambient temperature [18,19]. The adulterants caffeine and phenacetin, also used as cutting agents in nyaope, have previously been shown to be stable in methanol for at least 24 h of storage at ambient temperature [20]. The adulterant dextromethorphan has been reported to be stable in acetonitrile after 8 h of storage [21].

Long term storage of cannabis in ethanol extracts is known to lead to degradation of the active ingredient Δ^9-tetrahydrocannabinol to the oxidation product cannabinol [22] while diamorphine, on the other hand, hydrolyses to 6-monacetylmorphine and ultimately morphine due to interaction with water present in the solvent [23–25]. Fig. 1 shows the degradation products of Δ^9-tetrahydrocannabinol (Δ^9-THC) and heroin. Although methanol is believed to be a more efficient solvent for the extraction of cannabinoids [26] it, however, facilitates the hydrolysis of diamorphine to form 6-monacetylmorphine (6-MAM) [27]. Methanolic extracts, therefore, result in the immediate decomposition of diamorphine [28,29]. On the other hand, whilst chloroform is a suitable solvent for diamorphine since it dissolves the drug quantitatively over a wide concentration range, is compatible with instrumental analysis and does not degrade diamorphine, it is known to facilitate the breakdown of cannabinoids [29,30]. Some solvents may not be suitable for either diamorphine or Δ^9-THC because they do not dissolve the drugs quantitatively over a wide concentration range while others may be unsuitable for subsequent analysis. In short, a wide range of factors have to be taken into account in the choice of solvent for drug analysis.

Gas chromatography–mass spectrometry (GC–MS) has been widely used for the analysis of illicit drugs and nyaope is no exception [9,31–34]. GC–MS is suitable for the analysis of nyaope samples which contain both acidic and basic drugs that are volatile under GC–MS conditions without the need for derivatization. Suitable internal standards have included deuterated analogues as well as eicosane (C_{20}) and teracosane (C_{24}) [9,31–34].

This study seeks to identify a suitable solvent in which the majority of the components of nyaope can be dissolved prior to analysis by GC–MS and are stable under autosampler conditions. The solvents dichloromethane, ethanol, ethyl acetate, hexane, isopropanol and tertiary butyl alcohol were used for the extraction of the nyaope samples. These solvents were chosen since cannabinoids are easily soluble in most organic solvents and have been widely used to quantitatively dissolve both cannabinoids and opiates over a wide concentration range [9,22,31,35–37]. GC–MS was used for the analysis of samples with tetracosane (C_{24}) as the internal standard.

2. Material and methods

2.1. Chemicals

Dichloromethane (Distol-Pesticide residue grade) and hexane (HPLC grade) were purchased from Fischer Chemicals; absolute ethanol (Analytical reagent 99.9%) and tertiary butyl alcohol (ACS,

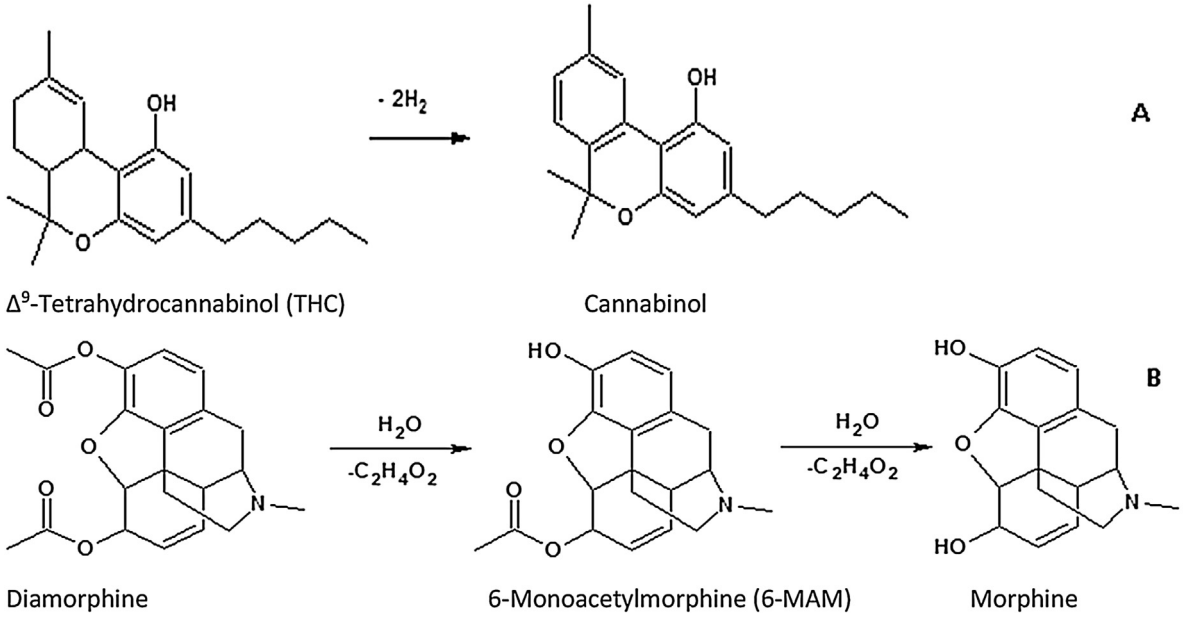

Fig. 1. (A) Degradation product of THC. (B) Degradation product of diamorphine.

Reag. Ph Eur) were purchased from Merck; ethyl acetate (Chromasolv for HPLC ≥ 99%) and tetracosane (99%) were purchased from Sigma-Aldrich; isopropanol (AR grade) was purchased from Associated Chemical Enterprise. Solvents were used as received without further purification. Efavirenz 600 mg tablets (PHD item 41047) and nevirapine 200 mg tablets (PHD item 41071) were both donated by Aspen Pharmacare. Cannabis and heroin street samples seized by the South African Police Services (SAPS) were used in this study.

2.2. Preparation of internal standards

The internal standard, tetracosane (C_{24}), was prepared at a final concentration of 0.02 mg/mL in each of the solvents under study i.e. dichloromethane (DCM), ethanol (ETOH), ethyl acetate (ETAC), hexane, isopropanol (i-PrOH) and tertiary butyl alcohol (t-BuOH). The internal standard solution was then used to dissolve the drug samples prior to the stability tests.

2.3. Sample preparation

Street cannabis and heroin samples seized by the SAPS were used to prepare simulated nyaope samples. The simulated samples were prepared by mixing the heroin street sample, cannabis street sample, efavirenz tablet sample and nevirapine tablet sample, to mimic as closely as possible a typical street nyaope sample. The heroin street samples were determined to contain caffeine, diamorphine, dextromethorphan, acetylcodeine, 6-monoacetylmorphine, noscapine, papaverine and phenacetin using GC–MS during routine case work at the SAPS Forensic Science Laboratory (SAPS-FSL).

The method used for the sample preparation is a modification of the methods reported in literature [37,38]. The samples were homogenised by grinding using a mortar and pestle. Homogenised samples ranging between 10 mg and 22 mg were mixed with 10 mL of the internal standard solution in a 20 mL head space vial. The mixture was sonicated for 15 min, filtered and the solution divided into seven 1 mL portions in amber GC–MS vials representing each of the time intervals. Each of the vials was analysed in triplicate without further dilution.

On the basis of the results for the simulated nyaope samples, tests were carried out on actual seized street samples of nyaope in selected solvents. Street samples were ground into a fine powder using a mortar and pestle. Separate aliquots of the homogenised street sample ranging from 10 mg to 16 mg were weighed into a 20 mL vial and mixed with 1 mL of each of the tertiary butyl alcohol, dichloromethane and isopropanol internal standard solution. The mixture was sonicated for 15 min, filtered and the solution divided into seven 100 μL portions in insert vials in the amber GC–MS vials representing each of the time intervals. Each of the vials was analysed in triplicate without further dilution.

To test the stability of the drugs in the solvent, samples were analysed after storage intervals of 0, 1, 6, 8, 24, 48 and 72 h at room temperature. The GC–MS analysis was performed using different vials for each time interval to minimise sample evaporation due to a perforated vial septum. The volume of sample in each vial was, however, kept the same.

2.4. Instrumentation

GC–MS analysis was carried out using an Agilent Technologies system (Chemetrix, RSA) consisting of a gas chromatograph (GC), Agilent 7890A, and mass selective (MS) detector (Agilent 5975 CVL MSD) with an auto sampler 7683 B series (1 μL injection). Chromatographic separation was performed on a computer controlled auto sampler used with a fused-silica capillary column HP-5MS (30 m × 0.25 mm, film thickness 0.25 μm; J&W Scientific, Folsom, CA, USA). Splitless injection was used at 280 °C. The GC oven temperature programme consisted of an initial temperature of 100 °C for 0.4 min, raised to 290 °C at a flow rate of 60 °C/min,

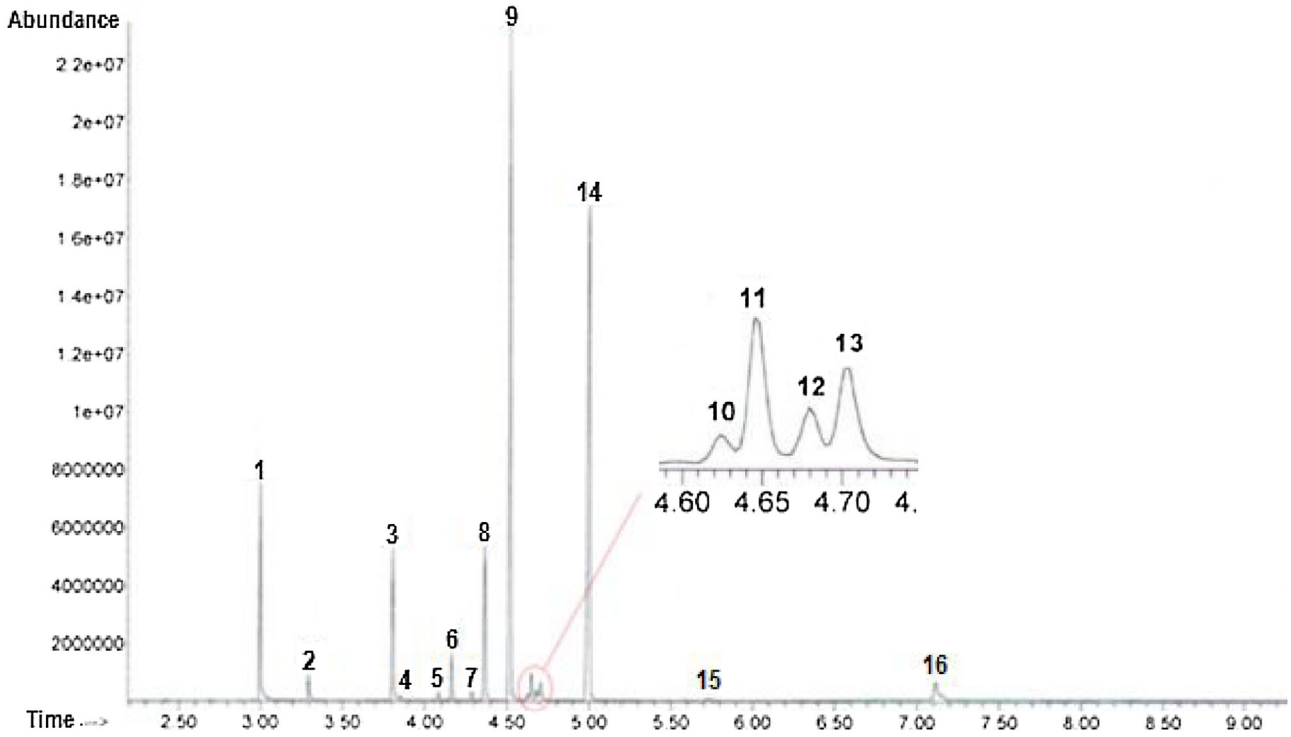

Fig. 2. Typical total ion chromatograph for the simulated nyaope samples. (1) Phenacetin; (2) caffeine; (3) EFV; (4) dextromethorphan; (5) tetrahydrocannabivarin; (6) tetracosane; (7) cannabidiol; (8) NVP; (9)Δ^9-THC; (10) cannabigerol; (11) acetylcodeine; (12) cannabinol; (13) 6-monoacetylmorphine; (14) diamorphine; (15) papaverine and (16) noscapine.

held at 290 °C for 2.4 min then raised to reach 316 °C at 60 °C/min and held for 3 min. The total run time was 9.40 min. High-purity helium (99.9995%) was used as the carrier gas, at a flow rate of 1 mL/min. The MS parameters used was performed as follows: the interface temperature (280 °C), the inlet temperature (250 °C), the ion-source temperature (230 °C), electron ionization (EI) at 70 eV and the mass spectrometer (quadrupole) used in scan mode. The spectra were recorded in the scan range of mass particles (m/z) from 35 to 550 amu, at a scan time of 1 scan/s (scan rate). Prior to analysis, confirmation that the instrument met QA standards was achieved using a system suitability test according to the SAPS-FSL protocol [39].

3. Results and discussion

The samples were successfully analysed using GC–MS in the presence of the internal standard tetracosane. Figs. 2 and 3 show typical chromatograms of the simulated nyaope sample and actual street nyaope sample, respectively. The peak areas of tetracosane and each of the target compounds nicotine, phenacetin, caffeine, EFV, dextromethorphan, tetrahydrocannabivarin, cannabidiol, NVP, Δ^9–THC, acetylcodeine, cannabinol, 6-monoacetylmorphine and diamorphine were determined from total ion chromatograms. The retention times (RT) of the individual components for the simulated nyaope samples as well as the components for actual seized street samples are given in Tables S1A and S2A respectively in the Supplementary information. Relative response ratios for each target compound in each replicate measurement were calculated by dividing the peak area of the target compound with the peak area of the internal standard [8]. The averages of these response ratios were determined and the normalised average response ratio was determined using the equation below:

$$R_N = ABS\left(100 - \frac{R_i}{R_0} \times 100\right)$$

where R_N is the normalised average response ratio, *ABS* is absolute value, R_i is the relative response ratio at the time $T_{i,}$ and R_0 is the relative response ratio for the initial injection. The ratio of cannabinol to Δ^9-THC was calculated by dividing the peak area response of cannabinol with the peak area response of Δ^9-THC. An increase in the ratio indicates an increase in cannabinol (degradation product) and a decrease in Δ^9-THC. The ratio of 6-MAM to diamorphine was calculated in a similar manner where an increase in the ratio indicates an increase in 6-MAM (degradation product) and a decrease in diamorphine. Table 1 summarises the results for the ratio of cannabinol to Δ^9-THC and 6-MAM to diamorphine, where 1 denotes a decrease of 0–15% and considered stable, 2 denotes a decrease of 15–30% and considered moderately stable and 3 denotes a loss above 30% and considered unstable [39]. Fig. 4 is a plot of normalized average response ratio versus time of 6-MAM to diamorphine and cannabinol to Δ^9-THC ratios for simulated street nyaope samples extracted with t-BuOH, DCM, ETAC, i-PrOH, ETOH and hexane respectively. Fig. 5 is a plot of normalized average response ratio versus time for EFV, NVP phenacetin, caffeine and dextromethorphan. The stabilities for the target compounds phenacetin, caffeine, EFV, dextromethorphan, tetrahydrocannabivarin, cannabidiol, NVP, cannabinol, Δ^9-THC, acetylcodeine, 6-MAM and diamorphine, in both simulated nyaope samples and actual street samples are summarised in Table S3A in the supplementary information similar to Table 1.

The results for individual solvents are discussed in the order of decreasing suitability for extraction and storage stability of the target compounds. Tertiary butyl alcohol, dichloromethane and isopropanol were selected for extraction and storage stability studies for the street samples of nyaope. The street sample tested was found to contain nicotine, α-caryophyllene, α-humelene, α/β-selinene, phenacetin, neophyltadiene, caffeine, palmitic acid, phytol, (*Z,Z,Z*)-9,12,15-octadecatrienoic acid, cannabivarol, 4,8,13-duvatriene-1,3-diol, cannabicyclol, tetrahydrocannabivarin,

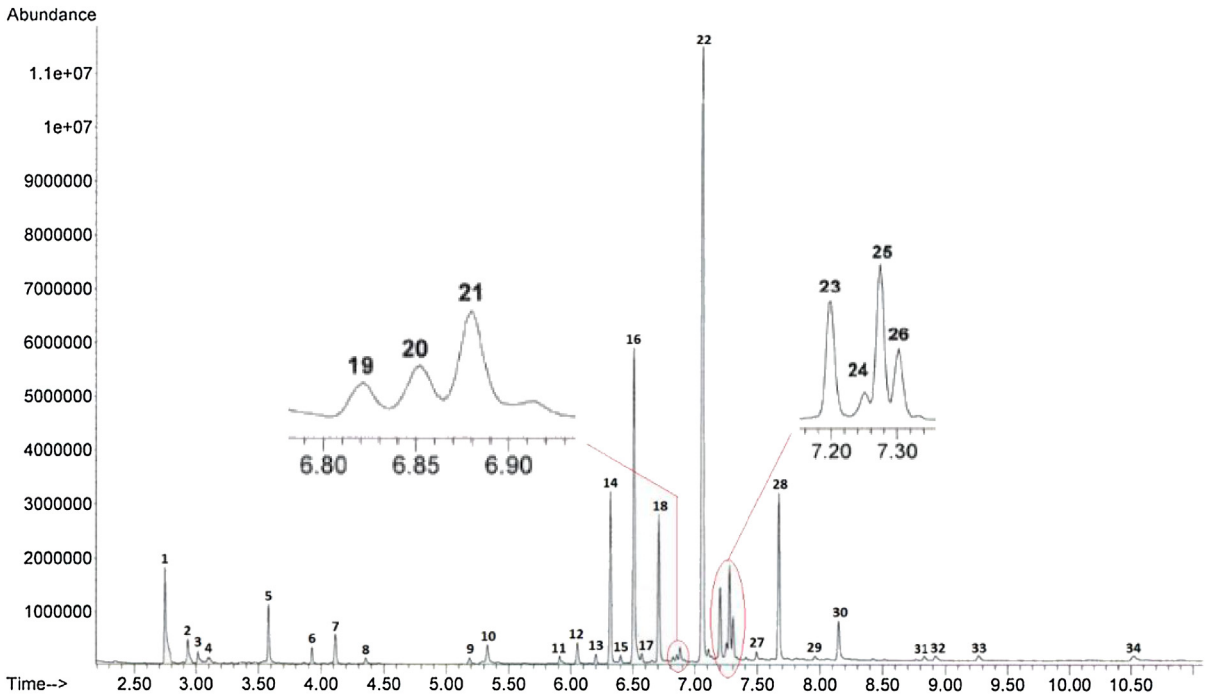

Fig. 3. Typical total ion chromatograph for an actual street sample of nyaope. (1) Nicotine; (2) α-caryophyllene; (3) α-humelene; (4) α/β-selinene; (5) phenacetin; (6) neophyltadiene; (7) caffeine; (8) palmitic acid; (9) phytol; (10) (*Z,Z,Z*)-9,12,15-octadecatrienoic acid; (11) cannabivarol; (12) 4,8,13-duvatriene-1,3-diol; (13) cannabicyclol; (14) tetrahydrocannabivarin; (15) cannabichromene; (16) internal standard tetracosane; (17) cannabivarin; (18) cannabidiol; (19) nevirapine; (20) cannabicoumaronone; (21) unknown (22); Δ^9-tetrahydrocannabinol; (23) cannabigerol; (24) acetylcodeine; (25) cannabinol; (26) 6-monoacetylmorphine; (27) heneicosane; (28) diamorphine; (29) squalene; (30) 11-butyl docosane; (31) unknown; (32) triacontane; (33) vitamin E and (34) β/γ-sitosterol.

Table 1
Summarised stabilities for the cannabinol to Δ^9-tetrahydrocannabinol and 6-monoacetylmorphine to diamorphine ratios.[a]

Time, h	Compound	Dichloromethane		Ethanol	Ethyl acetate	Hexane	Isopropanol		Tertiary butyl alcohol	
		Simulated sample	Street sample	Simulated sample	Simulated sample	Simulated sample	Simulated sample	Street sample	Simulated sample	Street sample
1	Cannabinol/Δ^9-THC	1	1	1	1	1	1	1	1	1
	6-MAM/ Diamorphine	1	1	1	1	1	1	1	1	1
6	Cannabinol/Δ^9-THC	1	2	1	1	1	1	1	1	1
	6-MAM/ Diamorphine	1	1	1	2	1	1	1	2	1
8	Cannabinol/Δ^9-THC	1	2	1	1	1	1	1	1	1
	6-MAM/ Diamorphine	1	1	1	2	1	1	1	2	1
24	Cannabinol/Δ^9-THC	1	2	1	1	1	1	1	1	1
	6-MAM/ Diamorphine	2	2	2	3	3	1	1	2	1
48	Cannabinol/Δ^9-THC	3	1	2	1	1	2	3	1	1
	6-MAM/ Diamorphine	1	1	2	3	2	3	3	2	1
72	Cannabinol/Δ^9-THC	3	1	1	2	1	2	3	1	1
	6-MAM/ Diamorphine	1	1	3	1	2	2	3	2	1

[a] The stabilities are summarised in Tables S1 and S3A in the Supplementary information, where 1 denotes a change of 0–15% and considered stable, 2 denotes a change of 16–30% and considered moderately stable and 3 denotes a change above 30% and considered unstable [40].

cannabivarin, cannabidiol, nevirapine, cannabichromene, cannabicoumaronone, Δ^9-tetrahydrocannabinol, cannabigerol, acetylcodeine, cannabinol, 6-monoacetylmorphine, diamorphine, vitamin E and β/γ-sitosterol. Diamorphine and Δ^9-tetrahydrocannabinol were identified on the basis of their retention time and mass spectral data using certified reference material. EFV, phenacetin, caffeine, cannabidiol, acetylcodeine, cannabinol, 6-monoacetylmorphine, and NVP were identified on the basis of their retention time and mass spectral data using USP reference standards. Nicotine, α-caryophyllene, α-humelene, α/β-selinene, neophyltadiene, palmitic acid, phytol, (*Z,Z,Z*)-9,12,15-octadecatrienoic acid, cannabivarol, 4,8,13-duvatriene-1,3-diol, cannabicyclol, tetrahydrocannabivarin, cannabivarin, cannabichromene, cannabicoumaronone, cannabigerol, vitamin E and β/γ-sitosterol were identified by comparing the experimental mass spectral data with the NIST mass spectral library (NIST 14).

A plot of the normalised relative response ratio (on the y-axis) vs injection time (on x-axis), for the ratio of 6-MAM to diamorphine and cannabinol to Δ^9-THC was prepared for the street samples extracted with each of the solvents tertiary butyl alcohol, dichloromethane and isopropanol as shown in Fig. 6. The ratios 6-MAM/diamorphine and cannabinol/Δ^9-THC evidence better stability of the nyaope components cannabinol, Δ^9-THC, 6-MAM and diamorphine during 72 h of storage when extracted with t-BuOH. They are next most stable in DCM and least stable when extracted with I-PrOH.

3.1. Tertiary butyl alcohol

Tertiary butyl alcohol was shown to be the best solvent in which the main components of nyaope are more stable as illustrated in Figs. 4–6. Stabilities shown in Table S1 as well as Table S3A in the Supplementary information indicate that the target compounds tetrahydrocannabivarin, cannabidiol, cannabinol, Δ^9-THC, 6-MAM and diamorphine are stable for 72 h of storage in both simulated and actual street nyaope samples. The ratios cannabinol to Δ^9-THC and 6-MAM to diamorphine confirm the stability of cannabinol, Δ^9-THC, 6-MAM and diamorphine during 72 h of storage. NVP and acetylcodeine are stable for 72 h of storage in simulated samples but fluctuate between instability and stability in actual street samples. Phenacetin and caffeine are stable for 72 h of storage in simulated samples while in the actual street samples, phenacetin is stable for 24 h of storage and caffeine is stable for 48 h of storage.

EFV, present only in simulated nyaope sample, was shown to be stable for 72 h of storage, while dextromethorphan is shown to fluctuate between instability and moderate stability. Nicotine, present only in actual street nyaope sample, was found to be stable for 72 h of storage. The fluctuation between instability and stability for acetylcodeine, NVP and dextromethorphan may be due to the fact that these components are present at a very low concentrations.

The stability of the target compounds in tertiary butyl alcohol is to be expected since it is an unreactive solvent [41,42]. From this it is clear that the compounds caffeine, phenacetin, EFV, NVP, Δ^9-THC, acetylcodeine, cannabidiol, cannabinol, diamorphine, tetrahydrocannabivarin and 6-MAM can be qualitatively extracted with tertiary butyl alcohol and analysed within 72 h.

3.2. Dichloromethane

The solvent that performed next best after tertiary butyl alcohol was dichloromethane. The results for the extraction of simulated nyaope samples as well as actual street nyaope samples with dichloromethane are shown in Table S1 as well as Table S3A in the

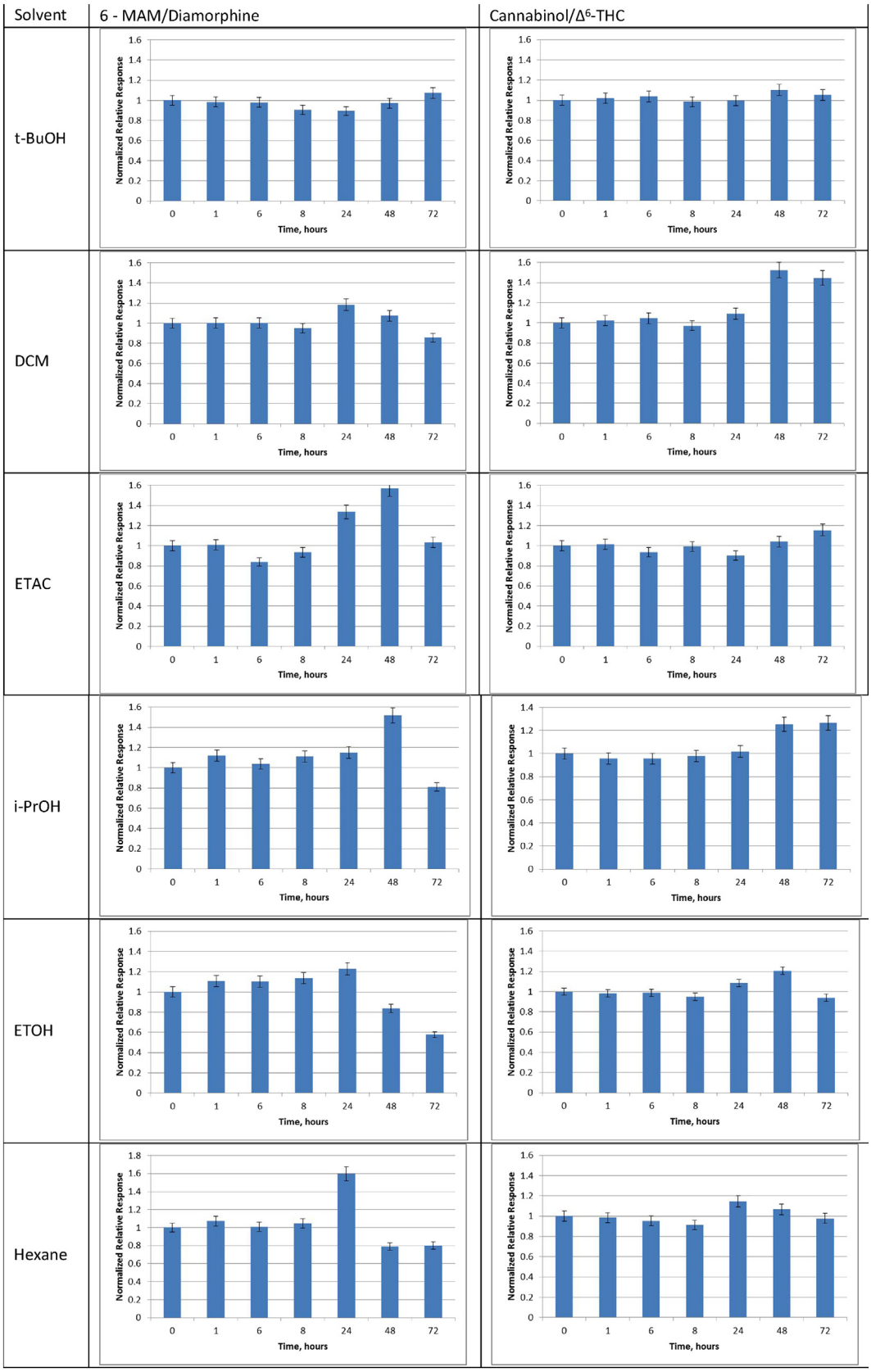

Fig. 4. Plot of normalized average response ratio vs time of 6-MAM/diamorphine and cannabinol/Δ^9-THC ratios for simulated street nyaope samples extracted with tertiary butyl alcohol, dichloromethane, ethyl acetate, isopropanol, ethanol and hexane respectively.

Fig. 5. Plot of normalized average response ratio vs time for EFV, NVP Phenacetin, Caffeine and Dextromethorphan for simulated street nyaope samples extracted with tertiary butyl alcohol, dichloromethane, ethyl acetate, isopropanol, ethanol and hexane respectively.

Supplementary information. The results for EFV, NVP, phenacetin, caffeine and dextromethorphan are illustrated in Fig. 5. In both simulated and actual street nyaope samples caffeine, tetrahydrocannabivarin, NVP, 6-MAM, diamorphine are shown to be stable for the 72 h of storage. The ratio 6-MAM to diamorphine shown in Figs. 4 and 6 confirms the stability diamorphine and 6-MAM in both simulated and actual street nyaope samples. Cannabinol and Δ^9-THC are shown to be stable for 72 h of storage in an actual street nyaope sample. Although cannabinol is shown to be stable for the 72 h of storage in simulated samples, the ratio of cannabinol to Δ^9-THC only confirms stability in actual street nyaope samples and shows both cannabinol and Δ^9-THC to be stable for 24 h of storage in simulated samples (Figs. 4 and 6). Dichloromethane contains hydrochloric acid that facilitates the decomposition of Δ^9-tetrahydrocannabinol to form cannabinol. Phenacetin is stable for 72 h in simulated samples while only stable for 24 h in the actual street nyaope sample. Cannabidiol and acetylcodeine are both stable for 72 h in actual street samples while acetylcodeine fluctuates between stability and instability and cannabidiol is only stable for 48 h in simulated samples. EFV, present only in simulated nyaope sample, was shown to be stable for 72 h of storage, while dextromethorphan is shown to be stable for 48 h of storage. Nicotine, present only in actual street samples, was found to be stable for 72 h of storage. As a highly volatile solvent, dichloromethane presents an additional problem in that it easily evaporates from the GC–MS sample vials [8].

3.3. Ethyl acetate

The results for the extraction of simulated nyaope samples with ethyl acetate are shown in Table S1 as well as Table S3A in the supplementary information. Ethyl acetate was found to be the next best solvent and performed as well as dichloromethane. The results for EFV, NVP, phenacetin, caffeine and dextromethorphan are illustrated in Fig. 5. The target compounds phenacetin, caffeine, tetrahydrocannabivarin, EFV, NVP, Δ^9-THC cannabinol 6-MAM and diamorphine are shown to be stable for 72 h of storage. The ratio of cannabinol to Δ^9-THC confirms the stability of cannabinol and Δ^9-THC, while the ratio of 6-MAM to diamorphine shows diamorphine and 6-MAM to fluctuate between instability and stability for 72 h of storage as shown in Fig. 4. 6-Monoacetylmorphine showed an increase after 24 h of storage while diamorphine also showed a slight decrease, which suggests that diamorphine undergoes hydrolysis in ethyl acetate. Ethyl acetate is hygroscopic and therefore absorbs moisture that facilitates the hydrolysis of diamorphine. Dextromethorphan is shown to be unstable after 48 h of storage.

3.4. Isopropanol

Isopropanol followed after ethyl acetate and dichloromethane and gave better stabilities than ethanol and hexane. The results for the extraction of simulated nyaope samples as well as actual street nyaope samples with isopropanol are shown in Table S1 as well as Table S3A in the Supplementary information. The results for EFV, NVP, phenacetin, caffeine and dextromethorphan are illustrated in Fig. 5. In both simulated and actual samples only tetrahydrocannabivarin is stable for 72 h of storage. Phenacetin, cannabidiol and acetylcodeine are stable in simulated samples for 72 h of storage whereas acetylcodeine and cannabidiol fluctuate between stability and instability in actual street samples. Phenacetin, caffeine and NVP are stable for 8 h of storage in actual street samples. While Δ^9-THC is shown to be stable for 72 h of storage in both simulated and actual street samples, the ratio cannabinol to Δ^9-THC only

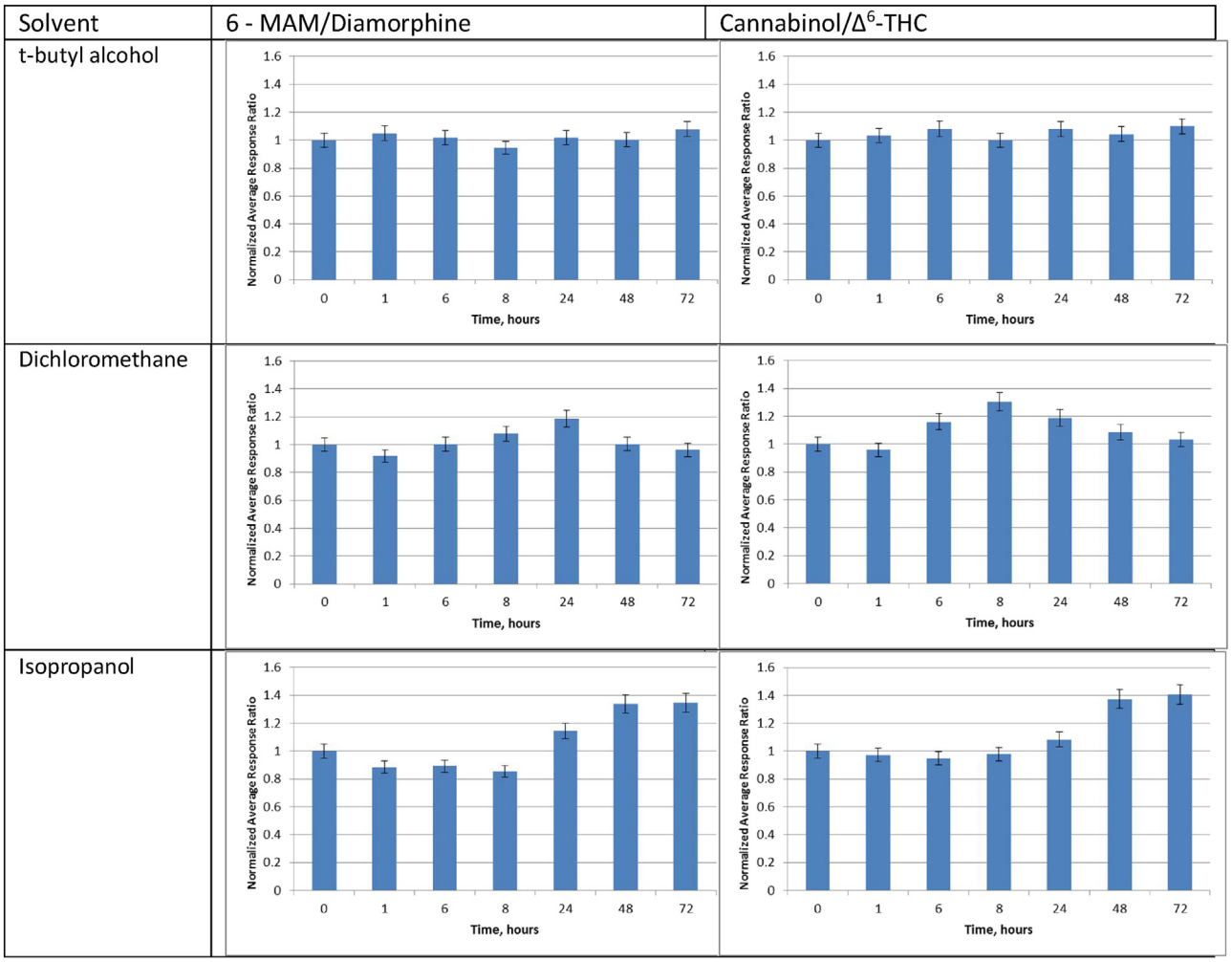

Fig. 6. Normalized average response ratios of 6-MAM to diamorphine and cannabinol to Δ^9-THC for the actual street samples.

confirms the stability for 72 h of storage in simulated samples and shows Δ^9-THC and cannabinol to be only stable for 24 h of storage in actual street samples (Figs. 4 and 6). The ratio 6-MAM to diamorphine shown in Table 1 and Fig. 4, shows 6-MAM and diamorphine to be unstable after 24 h of storage for the street samples. This is to be expected since the water found in isopropanol facilitates the hydrolysis of diamorphine. The target compound found only in simulated sample EFZ was found to be stable for 72 h of storage while dextromethorphan was found to be unstable after 24 h of storage. The target compound nicotine found only in the actual street nyaope sample was found to be stable after 8 h of storage.

3.5. Ethanol

Ethanol has previously been found to be a suitable solvent for the extraction of diamorphine [29]. However the water in ethanol will facilitate the hydrolysis of diamorphine to 6-MAM and subsequently morphine. The results for the extraction of simulated nyaope samples with ethanol are shown in Table S1 as well as Table S3A in the Supplementary information. The results for EFV, NVP, phenacetin, caffeine and dextromethorphan are illustrated in Fig. 5. The target compounds phenacetin, caffeine, EFV, tetrahydrocannabivarin, cannabidiol, NVP, Δ^9-tetrahydrocannabinol, acetylcodeine, cannabinol, 6-monoacetymorphine and diamorphine were found to be stable for 48 h of storage. The ratio 6-MAM to diamorphine confirms the stability of diamorphine and 6-MAM for 48 h of storage, while the ratio cannabinol to Δ^9-THC shows cannabinol and Δ^9-THC to be stable for 72 h of storage, shown in Fig. 4. Dextromethorphan was shown to be unstable after 24 h of storage.

3.6. Hexane

The results for the extraction of simulated nyaope samples with hexane are shown in Table S1 as well as Table S3A in the Supplementary information. The results for EFV, NVP, phenacetin, caffeine and dextromethorphan are illustrated in Fig. 5. Caffeine was the only compound shown to be stable after 72 h of storage. The target compounds phenacetin, EFV, tetrahydrocannabivarin, Δ^9-THC, acetylcodeine, cannabinol, and diamorphine are all stable to moderately up to 48 h of storage. The ratio cannabinol to Δ^9-THC shows cannabinol and Δ^9-THC to be stable for 72 h of storage, while the ratio 6-MAM to diamorphine shows diamorphine and 6-MAM to fluctuate between stability and instability. Cannabidiol was found to fluctuate between stability and instability for 72 h of storage, shown in Fig. 4. Dextromethorphan and NVP were shown to stable for 24 h of storage. Hexane is a non-polar solvent with low eluent strength and therefore not expected to extract the more polar diamorphine and 6-monoacetylmorphine. The extraction with non-polar hexane may be good for Δ^9-tetrahydrocannabinol but not suitable for the more polar diamorphine and its adulterants. Hexane was the solvent which exhibited the least stability for the target compounds.

4. Conclusions

This study has shown that the major components of nyaope are most stable in tertiary butyl alcohol stored in amber vials at room temperature. The majority of the target compounds were stable up to 72 h of autosampler stability for both simulated and actual street nyaope samples extracted with dichloromethane, ethyl acetate and tertiary butyl alcohol. Dichloromethane is a highly volatile solvent and therefore easily evaporates from the GC–MS sample vials, furthermore it contains hydrochloric acid that facilitates the decomposition of Δ^9-tetrahydrocannabinol to form cannabinol which makes tertiary butyl alcohol the more suitable solvent. In order of suitability for extraction and autosampler stability, tertiary butyl alcohol was the most suitable solvent followed by dichloromethane, ethyl acetate, isopropanol, ethanol, and lastly, hexane. With the exception of dextromethorphan all the components studied can be extracted with tertiary butyl alcohol, dichloromethane, ethyl acetate, isopropanol or ethanol provided instrumental analysis is performed within 48 h. This would allow for the identification quantification and profiling of nyaope using GC–MS. Samples prepared for the profiling of nyaope can therefore be extracted with tertiary butyl alcohol with subsequent instrumental analysis performed within 72 h of preparation. Using this data it is possible to identify the components of a nyaope sample and in principle establish possible links between drug seizures.

Credit author statement

P.M. Mthembi: conceptualization, methodology, investigation, writing — original draft. **E.M. Mwenesongole:** supervision, validation, writing, — review and editing. **M.D. Cole:** supervision, validation, writing, — review and editing.

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.2018.08.001.

References

[1] K.E. Mokwena, M. Huma, Experiences of 'nyaope' users in three provinces of South Africa, Afr. J. Phys. Health Educ. Recreat. Dance (Suppl. 1:2) (2014) 352–363.

[2] J. Masombuka, Children's Addiction to the Drug "NYAOPE" in Soshanguve Township: Parents' Experiences and Support Needs. Thesis 154 p, University of South Africa, 2013.

[3] D.J. Grelotti, E.F. Closson, J.A. Smit, Z. Mabude, L.T. Matthews, S.A. Safren, D.R. Bansberg, M.J. Mimiaga, Whoonga: potential recreational use of HIV antiretroviral medication in South Africa, AIDS Behav. 18 (2014) 511–518.

[4] K. Rough, J. Dietrich, T. Essien, D.J. Grelotti, D.R. Bansberg, G. Gray, I.T. Katz, Whoonga and the abuse and diversion of antiretrovirals in Soweto, South Africa, AIDS Behav. 18 (2014) 1378–1380.

[5] R. Thomas, S. Velaphi, Abuse of antiretroviral drugs combined with addictive drugs by pregnant women is associated with adverse effects in infants and risk of resistance, S. Afr. J. Child Health 8 (2014) 78–79.

[6] South African Police's Forensic Fact File no. 9 of 2013.

[7] A.A. Khine, K.E. Mokwena, M. Huma, L. Fernandes, Identifying the composition of street drug nyaope using two different mass spectrometer methods, Afr. J. Drug Alcohol Stud. 14 (2015) 49–56.

[8] L. Aalberg, K. Andersson, C. Bertler, M.D. Cole, Y. Finnon, H. Huizer, K. Jalava, E. Kaa, E. Lock, A. Lopes, A. Poortman-van der Meer, E. Sippola, J. Dahleń, Development of a harmonised method for the profiling of amphetamines. II. Stability of impurities in organic solvents, Forensic Sci. Int. 149 (2005) 231–241.

[9] C. Kuleya, S. Hall, L. Gautam, M.D. Cole, An optimised gas chromatographic–mass spectrometric method for the chemical characterisation of benzylpiperazine and 1-arylpiperazine based drugs, Anal. Methods 6 (2014) 156–163.

[10] J.A. Inciardi, H.L. Surratt, S.P. Kurtz, T.J. Cicero, Mechanisms of prescription drug diversion among drug-involved club- and street-based populations, Pain Med. 8 (2007) 171–183.

[11] F. Larkan, B. Van Wyk, J. Saris, Of remedies and poisons: recreational use of antiretroviral drugs in the social imagination of South African carers, Afr. Soc. Rev. 14 (2010) 62–73.

[12] Z.T. Shembe, The Effects of Whoonga on the Learning of Affected Youth in Kwa-Dabeka Township. Thesis, University of South Africa, 2013 123 p.

[13] N. Turjanski, G.G. Lloyd, Psychiatric side-effects of medications: recent developments, Adv. Psychiatr. Treat. 11 (2005) 58–570.

[14] S. Garda in 'Getting Help — Coping with Drugs. Department of Community safety. Gauteng Provincial Government. Republic of South Africa, 2011. Available from: http://www.gauteng.gov.za/government/departments/community-safety/Documents/drug%20abuse.pdf. (Accessed 30 April 2016).

[15] P. Lemmer, S. Schneider, M. Schuman, C. Omes, V. Arendt, J.C. Tayari, L. Fundira, R. Wennig, Determination of nevirapine and efavirenz in plasma using GC/MS in selected ion monitoring mode, Ther. Drug Monit. 27 (2005) 521–525.

[16] M. Vogel, N. Bertram, J.C. Wasmuth, J. Emmelkamp, J.K. Rockstroh, C. Reichel, Determination of nevirapine in plasma by GC–MS, J. Chromatogr. Sci. 48 (2010) 91–94.

[17] M.B. Gatch, A. Kozlenkov, R.-Q. Huang, W. Yang, J.D. Nguyen, J. Gonza'lez-Maeso, K.C. Rice, C.P. France, G.H. Dillon, M.J. Forster, J.A. Schetz, The HIV antiretroviral drug efavirenz has LSD-like properties, Neuropsychopharmacology 38 (2013) 2373–2384.

[18] N.L. Rezk, R.R. Tidwell, A.D.M. Kashuba, Simple and rapid quantification of the non-nucleoside reverse transcriptase inhibitors nevirapine, delavirdine, and efavirenz in human blood plasma using high-performance liquid chromatography with ultraviolet absorbance detection, J. Chromatogr. B 774 (2002) 79–88.

[19] B.S. Kappelhoff, H. Rosing, A.D.R. Huitema, J.H. Beijnen, Simple and rapid method for the simultaneous determination of the non-nucleoside reverse transcriptase inhibitors efavirenz and nevirapine in human plasma using liquid chromatography, J. Chromatogr. B 792 (2003) 353–362.

[20] L.C.S. Sena, H.R. Matos, H.S. Dórea, M.F. Pimentel, D.C.A.S. de Santana, F.J.M. de Santana, Dispersive liquid–liquid microextraction based on solidification of floating organic drop and high-performance liquid chromatography to the analysis of cocaine's major adulterants in human urine, Toxicology 376 (2017) 102–112.

[21] T. Goyal, A novel stability indicating HPLC method for simultaneous estimation of guaiphenesin: chlorpheneramine maleate & dextromethorphan HBr, Int. J. Pharm. Sci. Res. 4 (2013) 4435–4441.

[22] M. Carbone, F. Castelluccio, A. Daniele, A. Sutton, A. Ligresti, V. Di Marzo, M. Gavagnin, Chemical characterisation of oxidative degradation products of Δ^9-THC, Tetrahedron 66 (2010) 9497–9501.

[23] D.A. Barrett, A.L.P. Dyssegaard, P.N. Shaw, The effect of temperature and pH on the deacetylation of diamorphine in aqueous solution and in human plasma, J. Pharm. Pharmacol. 44 (1992) 606–608.

[24] A.R.L. Wijesekera, D.M.U.J. Abeysinghe, K.C. Pathirana, Studies on the degradation of heroin, Forensic Sci. Int. 67 (1994) 147–154.

[25] R. Brenneisen, F. Hasler, GC/MS determination of pyrolysis products from diacetylmorphine and adulterants of street heroin samples, J. Forensic Sci. 47 (2002) 1–4.

[26] R.N. Smith, C.G. Vaughan, The decomposition of acidic and neutral cannabinoids in organic solvents, J. Pharm. Pharmacol. 29 (1971) 286–290.

[27] H. Huizer, A.J. Poortman, Some Aspects of the Gas Chromatographic (GC) Analysis of Heroin. United Nations Scientific and Technical Notes. SCITEC/5, 1989. Available from: https://www.unodc.org/pdf/scientific/SCITEC_5.pdf. (Accessed 2 June 2016).

[28] K.M. Varshney, HPTLC study of the stability of heroin in methanol, J. Planar. Chromatogr. Mod. TLC 15 (2002) 46–49 Available from: https://www.researchgate.net/publication/245404751_HPTLC_study_of_the_stability_of_-heroin_in_methanol. (Accessed 4 December 2017)..

[29] L. Hong, Z. Wen-song, F. Ping, Y. Hong-mei, J. De-qiang, L. Shao-peng, L. Zhi-qiang, L. Can-peng, The study on the stability of heroin standard in organic solvents (abstract), Forensic Sci. Technol. 39 (2014) 3.

[30] C.E. Turner, J.T. Henry, Constituents of *Cannabis sativa* L. IX: stability of synthetic and naturally occurring cannabinoids in chloroform, J. Pharm. Sci. 64 (1975) 357–359.

[31] UNODC, in: S. Section (Ed.), Methods for Impurity Profiling of Heroin and Cocaine, United Nations, Vienna, 2005.

[32] G. Suurkuusk, Validation for the Gas Chromatographic Method for THC, CBD and CBN Determination. Thesis 40 p, University of Tartu, 2010.

[33] E.M. Mwenesongole, L. Gautam, S.W. Hall, J.W. Waterhouse, M.D. Cole, Simultaneous detection of controlled substances in waste water, Anal. Methods 5 (2013) 3248–3254.

[34] R. Ruchlemer, M. Amit-Kohn, D. Raveh, L. Hanuš, Inhaled medicinal cannabis and the immunocompromised patient, Support. Care Cancer 23 (2015) 819–822.

[35] D.R. Morello, S.D. Cooper, S. Panicker, J.F. Casale, Signature profiling and classification of illicit heroin by GC–MS analysis of acidic and neutral manufacturing impurities, J. Forensic Sci. 55 (2010) 42–49.

[36] P. Kintz, C. Jamey, V. Cirimele, R. Brenneisen, B. Lude, Evaluation of acetylcodeine as a specific marker of illicit heroin in human hair, J. Anal. Toxicol. 22 (1998) 425–429.

[37] U.K. Ahmad, Y. Muniandy, M.S. Hassan, Physical analysis and chemical profiling of illicit herbal cannabis using multivariate analysis, Malays. J. Forensic Sci. 5 (2005) 26–34.

[38] UNODC, in: S. Section (Ed.), Recommended Methods for the Identification and Analysis of Cannabis and Cannabis Products, United Nations, Vienna, 2009.

[39] SAPS-Forensic Science Laboratory: Procedure Manual: Chemistry Section — Maintenance and monitoring of GC–MS instrumentation. Revision 07, 2017.
[40] E. Saar, D. Gerostamoulos, O.H. Drummer, B. Beyer, Assessment of the stability of 30 antipsychotic drugs in stored blood specimens, Forensic Sci. Int. 215 (2012) 152–158.
[41] Product information Chemindustry.ru available at: http://chemindustry.ru/tert-Butanol.php. (Accessed 28 December 2017).
[42] W. Reeve, C.M. Erikson, P.F. Aluotto, A new method for the determination of the relative acidities of alcohols in alcoholic solutions. The nucleophilicities and competitive reactivities of alkoxides and phenoxides, Can. J. Chem. 57 (1979) 2747–2754.