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DATES:

Received: 07 Aug. 2020

Revised: 15 Apr. 2021

Accepted: 04 July 2021

Published: 29 Nov. 2021

HOW TO CITE:

Mthemb PM, Mwenesongole EM, Cole MD. A validated method for the analysis and profiling of 'nyaope' using gas chromatography – mass spectrometry. *S Afr J Sci.* 2021;117(11/12), Art. #8738. <https://doi.org/10.17159/sajs.2021/8738>

ARTICLE INCLUDES:

- Peer review
- Supplementary material

DATA AVAILABILITY:

- Open data set
- All data included
- On request from author(s)
- Not available
- Not applicable

EDITOR:

Priscilla Baker

KEYWORDS:

heroin, cannabis, nyaope, GC–MS, sample comparison

FUNDING:

University of the Free State

A validated method for the analysis and profiling of 'nyaope' using gas chromatography – mass spectrometry

Nyaope, a Tswana word for a mixture or 'mish-mash', describes a drug cocktail consisting of heroin, cannabis, and on occasion other controlled substances and warfarin. It is highly addictive with extremely unpleasant side effects caused by withdrawal from the drug. It is a problem drug especially in townships in South Africa. However, its prevalence in neighbouring southern African states and further afield is not yet known. There is currently no validated method for the analysis and comparison of nyaope. We describe a validated method for the gas chromatography – mass spectrometry analysis of nyaope so that within-batch and between-batch comparisons of nyaope can successfully be made for the first time. The validated method managed an accuracy within the range 80–120%, the precision was less than 20% for all analytes and managed linearity with $R^2 \geq 0.99$. The detection limits for diamorphine, efavirenz, nevirapine and Δ^9 -tetrahydrocannabinol were 14.2, 18.6, 18.7 and 9.94 pg on column, respectively, and the limits of quantitation were 43.1, 56.3, 56.6 and 30.1 pg on column, respectively. The simulated and casework samples were successfully discriminated into original batches using the identified nyaope components, the unsupervised chemometric methods principal component analysis and hierarchical clustering, as well as chromatographic profiles.

Significance:

- A validated method for the analysis and comparison of nyaope allows for data exchange between law enforcement agencies in South Africa and, provided the appropriate quality control measures are in place, between South Africa, neighbouring states and countries further afield. Additionally, public health measures can be put in place now that it is possible to use a validated method to determine the contents of nyaope.

Introduction

Despite the first reports being in the early 2000s^{1,2}, there remains a dearth of analytical chemistry and forensic science literature on the drug 'nyaope'. Nyaope, the name of which is derived from the Tswana word for mixture or 'mish-mash'², is reported to be a mixture of powdered heroin, herbal cannabis and prescription antiretrovirals. It has also been reported to contain methamphetamine and warfarin, although some of these claims are disputed. An unpublished study reports a much wider range of drugs found in nyaope samples.³ In parts of South Africa, it is known as 'Woonga' (spelled 'Wunga' in Zulu).² It has no single composition and mixtures follow trends⁴, although the predominant drug by mass is cannabis, followed by heroin. It is typically smoked after being mixed with tobacco.

Nyaope contains substances that have been controlled by the *South African Drug and Drug Trafficking Act* since 1992. The name nyaope itself is a street name and therefore cannot be listed in the Act. It is inexpensive, with a single dose costing ZAR20.00–30.00 (circa USD2), and is widely used by the coloured and black communities in South African townships, by addicts who are sometimes as young as 12 or 13 years old.⁴ In a conversation with a colleague from the Portuguese criminal police (Alegre J 2019, oral communication, May 08), it was established that, although the drug is widely reported in South Africa, nothing is known of its distribution in neighbouring Mozambique, Zimbabwe, Botswana and Namibia, although there are anecdotal reports of its use in Angola. Because of the severe form that the addiction to nyaope can take, as well as the intensity and nature of the withdrawal symptoms, the social cost of this drug is enormous⁵ and addicts, including children, adopt chaotic lifestyles, such as dropping out of school and engaging in criminal activity including theft and prostitution, to fund their drug taking⁴.

The health risks associated with using nyaope are not well investigated but reports include restriction of growth and development in neonates⁶ and infective endocarditis⁷ which had been misdiagnosed elsewhere as pulmonary tuberculosis or pneumonia⁸. The vast majority of users are HIV positive.⁷ Other problems associated with nyaope use include damaged and infected veins, damaged heart valves, tissue infections, liver failure, kidney disease and lung problems.⁹ It therefore represents a significant public health risk.

In South Africa, at present, the criminal law punishes drug-related offences by a fine or imprisonment. The scale of the nyaope problem is difficult to quantify. At present, there are few representative surveys on drug use and abuse in South Africa. A recent policy brief summarises the rise in trafficking and abuse of heroin in South Africa.¹⁰ However, there is a growing thesis that to tackle the drug problem in South Africa, including that associated with nyaope, a number of approaches need to be taken.⁴ These approaches include (1) punishment of those manufacturing, trafficking and distributing the drug. Additionally, a forensic care process is proposed towards addressing the nyaope problem where (2) drug users are properly catered for by rehabilitation schemes, (3) the social circumstances of the drug users are changed and (4) a Public Health Awareness Scheme is implemented.⁴ Of course, these approaches could be applied to many drug problems across the globe.

In order that (1) and (4) can be achieved and supported, it is necessary to prove the identity of the drug as nyaope and then to identify and quantify the drug contents. In order to achieve this identification, a validated analytical method for the analysis of the contents of and comparison of nyaope is required – to date no such method has been developed. This is further exacerbated by the fact that the usual methods for cannabis analysis cause heroin to break down, and, conversely, those used for heroin cause cannabis to break down. Our previous studies have identified that two forensic science questions need to be identified before police operations involving nyaope commence¹¹: how should nyaope be stored post-seizure¹¹, and how should the drug be prepared prior to analysis by gas chromatography – mass spectrometry (GC–MS)¹². In this paper, we present, for the first time, a validated analytical method for the identification of nyaope and quantification of the drug components, which addresses the analysis of heroin and cannabis when contained in the same drug sample. We further demonstrate that, with correct collection, storage and sample preparation, it is possible to compare nyaope samples, identify those which are related and discriminate between those that are not, using, for the first time, chemometric clustering techniques. How this method will assist law enforcement and public health officials in South Africa, and further afield, is also discussed.

Materials and methods

Chemicals

Tertiary butyl alcohol (t-BuOH) was purchased from Merck (Darmstadt, Germany), tetracosane 99% was purchased from Sigma-Aldrich (St Louis, MO, USA), isopropanol (i-PrOH)-AR grade was purchased from Associated Chemical Enterprise (Johannesburg, South Africa). Representative compounds, identified in casework samples of nyaope by the South African Police Service (SAPS), were used to validate the GC–MS method. Certified reference standards of Δ^9 -tetrahydrocannabinol (Δ^9 -THC) and diamorphine (both 1 mg/mL) were purchased from Cerilliant-Sigma Aldrich (Austin, TX, USA). Caffeine and phenacetin were purchased from the US-Pharmacopeia (Rockville, MD, USA) as USP powder reference standards while efavirenz and nevirapine were purchased from WHO International Chemical Reference Substances (Strasbourg, France) as ICRS powder reference substances.

Preparation of internal standards

The internal standard solution, tetracosane (C_{24}), was prepared at a final concentration of 0.02 mg/mL in tertiary butyl alcohol. Tertiary butyl alcohol has previously been shown to be the solvent of choice for presenting nyaope extracts to the GC–MS.¹² The internal standard solution was used to dilute the certified reference standards, and other samples, before GC–MS analysis.

Preparation of calibration standards

Stock solutions (1 mL at 1 mg/mL) of Δ^9 -THC in methanol and diamorphine in acetonitrile were placed in an amber GC–MS vial, evaporated to dryness under nitrogen and then re-dissolved in 1 mL of the internal standard solution to give stock solutions of 1 mg/mL. Phenacetin, caffeine, efavirenz and nevirapine were dissolved at concentrations of 1.03, 1.00, 0.998 and 1.05 mg/mL, respectively, in the internal standard solution. From these, 14 standards in the concentration range 0–1.0 mg/mL, at notional concentrations of 0, 0.001, 0.0025, 0.005, 0.0075, 0.01, 0.025, 0.05, 0.075, 0.1, 0.25, 0.5, 0.75 and 1.0 mg/mL were prepared.

Instrumentation

GC–MS analysis was carried out using an Agilent Technologies system (Chematrix, RSA) consisting of a gas chromatograph, Agilent 7890A, and mass selective detector (Agilent 5975 CVL MSD) with an auto sampler 7683 B series (1 μ L injection). Chromatographic separation was performed using a computer-controlled autosampler with a fused-silica capillary column HP-5MS (30 m \times 0.25 mm i.d., film thickness 0.25 μ m; 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 rate of

60 °C/min, 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 9.4 min. High-purity helium (99.9995%) was used as the carrier gas, at a flow rate of 1 mL/min. The MS parameters used were as follows: the interface temperature was 280 °C, the inlet temperature 250 °C, the ion-source temperature 230 °C, electron ionisation was achieved with a source voltage of 70 eV and the mass spectrometer (quadrupole) was used in scan mode. The spectra were recorded in the scan range (m/z) 35 to 550 amu, at a scan rate of 1 scan/s.

Method validation

The method was validated by determining the precision of the retention index of each compound, the linearity of detector response, the limit of detection and of quantitation, repeatability and the reproducibility of the measurements.^{13,14} The precision of the retention index was obtained for each compound (phenacetin, caffeine, efavirenz, nevirapine, Δ^9 -THC and diamorphine) by calculating the mean, standard deviation and relative standard deviation of the retention index, relative to tetracosane, for 10 replicate analyses.

Linearity of the detector response to the exemplar drugs was determined by preparation of calibration curves for samples in the concentration range 0.00–1.00 mg/mL. The regression equations for detector response relative to the internal standard, the value of R^2 and residual plot analysis were used to confirm linearity of detector response.

Limit of detection (LOD) and limit of quantitation (LOQ) were determined by the calibration curve slope using reference sample solutions with concentrations in the vicinity of the LOD¹⁵, namely 0.000, 0.001, 0.0025, 0.005, 0.01 and 0.05 mg/mL and the equations:

$$LOD = \frac{3.3 \times \sigma \times C_{IS}}{s} \quad \text{Equation 1}$$

$$LOQ = \frac{10 \times \sigma \times C_{IS}}{s} \quad \text{Equation 2}$$

where σ = standard error of the measured response, S = slope of the regression line and C_{IS} = concentration of the internal standard = 0.018 mg/mL

To measure the accuracy of the method (closeness to true concentration), 10 replicate measurements of standards of known concentrations were made, the experimental concentrations determined and Equation 3 applied:

$$\% \text{ Accuracy} = \frac{\text{Measured concentration}}{\text{actual concentration}} \times 100 \quad \text{Equation 3}$$

Precision is a measure of the closeness of the analytical results obtained from a series of replicate measurements of the same measure under the conditions of the method. Intra-assay precision (repeatability) and inter-assay precision (reproducibility) were assessed using drug standard mixtures of phenacetin, caffeine, efavirenz, nevirapine, Δ^9 -THC and diamorphine at three concentration levels (0.01, 0.1 and 1.00 mg/mL). Repeatability was assessed by making 10 replicate analyses of the drug standards at three concentration levels and calculating the mean, standard deviation and relative standard deviation of the relative response to the internal standard.

Reproducibility was assessed by making five replicate analyses of the drug standards over five consecutive days at the three concentration levels, and calculating both within group (W) and between group (B) precision using one-way ANOVA (Group = Day)¹⁶:

$$\%RSD_w = \frac{\sqrt{MSW}}{x} \times 100 \quad \text{Equation 4}$$

$$\%RSD_B = \frac{\sqrt{(MSB - MSW)/n}}{x} \times 100 \quad \text{Equation 5}$$

If $MSB < MSW$, set $\%RSD_B = 0$

where RSD = relative standard deviation, \bar{X} = grand mean of all observations, n = number of observations in group, MSW = mean of squares within group, MSB = mean of squares between groups.

Nyaope sample profiling and comparison

In order to investigate the validity of the method for nyaope sample identification and comparison, both simulated samples and casework samples of nyaope were analysed.

Street cannabis and heroin samples seized by the SAPS were used to prepare simulated nyaope samples. Three blind simulated samples were prepared by mixing a heroin street sample, a cannabis street sample, efavirenz tablets and nevirapine tablets, in different combinations and proportions, to mimic as closely as possible a typical street nyaope sample. The three mixtures were homogenised by grinding using a mortar and pestle. The samples were then further divided into six blind sub-samples each to give a total of 18 samples marked S1–S18. Homogenised samples which had a mass ranging between 12 mg and 14 mg were mixed with 3 mL of the internal standard solution (0.02 mg/mL tetracosane in tertiary butyl alcohol) in a 20-mL head space vial. The mixture was sonicated for 15 min^{12,17,18}, filtered into amber GC–MS vials and analysed in triplicate. Each of the extracts of the simulated samples S1–S18 was analysed at 0, 24, 48 and 72 h in order to confirm the stability of the extract once prepared.¹³ Additionally, chromatograms of members of each of the three groups were compared at the same time intervals to determine whether samples from the same parent batch could be discriminated after these time intervals. Finally, chromatograms from one member of each of the three groups were compared at these time intervals to demonstrate whether it is possible to discriminate between groups.

Five casework samples of nyaope were ground into a fine powder using a mortar and pestle. Sub-samples (circa 12–14 mg) of these street samples were placed in a 20-mL vial and extracted with 3 mL of the internal standard solution prior to analysis. Each of the casework samples was analysed in triplicate by GC–MS at $t=0$ after extraction. The chromatograms were compared to determine whether it was possible to discriminate between street samples. Each extract was then analysed after 24, 48 and 72 h to demonstrate stability of extracts of such samples. Semi-quantitation was conducted on caffeine, diamorphine and Δ^9 -THC for each of the five casework samples.

Two unsupervised chemometric methods – agglomerative hierarchical cluster analysis (HCA) and principal component analysis (PCA) – were performed on both the blind simulated and casework nyaope samples using the XLSTAT statistical and data analysis solution 2019 version. The HCA and PCA analysis were conducted for the samples analysed at 0, 24, 48 and 72 h.

Results and discussion

Compound identification – stability of retention indices

It is important that retention indices are stable for a given analytical method if drug comparisons are to be made. The retention indices were evaluated over five days for each component. The stability data for

representative components of nyaope are given in Table 1. The ANOVA test (single factor) demonstrated ($F_{\text{calc}} = 0.029$, $F_{\text{crit}} = 4.965$) that there was no significant difference between days and that the retention indices were stable.

The relative standard deviation (Table 1) for the retention indices of the compounds caffeine, diamorphine, efavirenz, nevirapine, phenacetin and Δ^9 -THC were all below 0.025%, further illustrating the stability of this parameter. Identification of components of nyaope can therefore be made on the basis of retention index and the mass spectrum of each separated compound.

Table 1: Stability data for retention indices of representative components of nyaope measured by gas chromatography – mass spectrometry

Compound	Average	SD	%RSD
Phenacetin	0.666	0.000148	0.022
Caffeine	0.835	0.000169	0.020
Efavirenz	0.835	0.000169	0.020
Tetracosane	1.000	0.000000	0.000
Nevirapine	1.096	0.000209	0.019
Δ^9 -Tetrahydrocannabinol	1.178	0.000137	0.012
Diamorphine	1.361	0.000332	0.024

Linearity of detector response

The detector response to standard compounds was linear over the concentration range investigated. The regression equations and R^2 -values are given for each of the components of nyaope measured (Table 2). All of the R^2 -values are above 0.99. This and analysis of residuals demonstrate that the detector response to these drugs is linear.

Limits of detection and quantitation

The detection limits and the limits of quantitation using this analytical method were determined as mass of the free drug on column and are given in Table 2. The detection limits varied between 9.94 pg and 39.1 pg on column and the limit of quantitation between 30.1 pg and 118 pg on column. The method is sufficiently sensitive to both detect these drugs in nyaope and quantitate them in a street sample.

Accuracy

Ten replicate analyses were performed for each drug at each of three known concentrations and the percentage accuracy of the measurement was determined. Accuracy figures were found to lie between 82% and 112%. These lie between the limits of 80% and 120% and are therefore considered accurate¹⁹⁻²¹, further validating the method.

Table 2: Regression equations, R^2 -values, limits of detection (LOD) and limits of quantitation (LOQ) for the exemplar drugs in nyaope

Drug	Regression equation	R^2 -value	LOD (pg)	LOQ (pg)
Caffeine	$y=23.94x+0.3923$	0.9975	21.0	63.6
Diamorphine	$y=31.36x+0.3186$	0.9981	14.2	43.1
Efavirenz	$y=30.92x+0.1483$	0.9995	18.6	56.3
Nevirapine	$y=29.49x+0.1673$	0.9987	18.7	56.6
Phenacetin	$y=28.05x+0.3406$	0.9970	39.1	118
Δ^9 -Tetrahydrocannabinol	$y=40.03x+0.499$	0.9951	9.94	30.1

Repeatability and reproducibility

Repeatability was assessed through 10 replicate analyses of the drugs at each of three different concentrations. The relative standard deviations of all analyses were found to lie below or at 15% (with nevirapine just above 15%, 0.01 mg/mL, RSD = 15.78%), demonstrating that the method is repeatable.^{22,23}

Reproducibility was assessed through five replicate analyses of the drug standards. The relative standard deviations for both within-group and between group precision were found to lie below 15% (with caffeine and nevirapine just above 15%, 0.01 mg/mL, RSD = 15.01% and 15.19% respectively) demonstrating that the method is reproducible.^{14,19,20}

On the basis of the data described above, and the recommendation of the UNODC that cannabis and heroin can be analysed by GC–MS, without derivatisation^{18,24}, the method was deemed suitable for the analysis of the principal drug types in nyaope.

Nyaope sample profiling and comparison

Each of the simulated samples was analysed after 0, 24, 48 and 72 h. The average peak area ratio (PAR) was determined for each of the simulated samples. The ANOVA of one of the samples gave $F_{\text{calc}} = 0.0106 < F_{\text{crit}} = 2.798$, demonstrating that there was no significant difference between the PAR over the 72 h. Retention time data are provided in Table 3. Pooled average response ratios for each of the batches were determined by averaging the PAR at t = 0, 24, 48 and 72 h. ANOVA of the samples from one of the batches using the F-test (single factor) gave $F_{\text{calc}} = 0.0268 < F_{\text{crit}} = 2.342$, demonstrating that there were no significant differences amongst the PARs for the samples belonging to the same batch over the 72 h autosampler storage.

Table 3: Retention time and relative retention time for the compounds identified in the analysis of simulated and casework nyaope samples. Identifications were made on the basis of retention indices and mass spectra of standards and casework samples (data available from corresponding author)

	Retention time	Relative retention time
Nicotine	2.411	0.444
Caryophyllene	2.582	0.476
Bulnesol	3.098	0.571
Phenacetin	3.133	0.577
Acetaminophen	3.194	0.589
Caffeine	3.535	0.651
Efavirenz	4.545	0.837
Methaqualone	4.748	0.875
Cocaine	4.891	0.901
Tetrahydrocannabivarin	5.241	0.966
Tetracosane	5.427	1.00
Cannabivarin	5.573	1.027
Cannabichromene	5.731	1.056
Cannabidiol	5.754	1.060
Nevirapine	5.949	1.096
Δ^9 -Tetrahydrocannabinol	6.234	1.149
Cannabigerol	6.414	1.182
Acetylcodeine	6.501	1.198
Cannabinol	6.518	1.201
6-Monoacetylmorphine	6.571	1.211
Diamorphine	7.020	1.293
Nonacosane	7.517	1.385
Vitamin E	8.686	1.601

From the data obtained, it can be demonstrated that each compound of interest can be identified, and that their relative proportions do not change over a 72-h period once extracted from nyaope into tertiary butyl alcohol, thus confirming previous work.¹² When each of the six samples for the three different simulated samples was analysed, it was found that the PAR, relating to each batch, could not be separated. It is therefore now possible to relate samples of nyaope to each other when they have come from a once larger parent batch, as demonstrated by the PAR.

When separated by GC–MS, it was possible to differentiate between the three batches of simulated nyaope (Figure 1) on the basis of the chromatographic profile. This method of extraction and analysis allows, for the first time, analysis and comparison of nyaope samples by a forensic science laboratory.

To demonstrate that the method could be applied to casework samples, five samples of nyaope (denoted as 2514202B, 2520902B, 3400002B, 37959902B and 50390902B) were analysed. Figure 2 is a typical total ion chromatograph for one of the casework samples (37959902B). The components identified in the other casework samples are summarised in Table 4. The components – cocaine, diamorphine, methaqualone and Δ^9 -THC – were identified on the basis of their retention time and mass spectral data using certified reference material. Caffeine was identified on the basis of retention time and mass spectral data using USP reference standards. Acetaminophen, acetylcodeine, bulnesol, cannabichromene, cannabicumaronone, cannabidiol, cannabigerol, cannabinol, cannabivarin, codeine, caryophyllene, 6-monoacetylmorphine, nicotine, nonacosane, tetrahydrocannabivarin and vitamin E were identified by comparing the experimental mass spectral data with the NIST mass spectral library NIST 14. From the components identified, it can be seen that it is possible to discriminate between street samples of nyaope and that the method described can be applied to forensic casework.

Semi quantitation of the five casework samples is summarised in Table 5, which shows the concentrations of the components caffeine, diamorphine and Δ^9 -THC. The caffeine concentration for samples 2514202B and 2520902B was below the limit of quantitation (63 μg on column). The pooled average concentration for each time interval of the three sub-samples in a batch was used to calculate the average %RSD, shown in Table 5, in order to determine if the concentrations were significantly different. The average %RSD was found to be <10% for all the components (caffeine, diamorphine and Δ^9 -THC) in the samples. This indicates that there was no significant difference between the concentrations of a particular component, over a period of 72 h once extracted into tertiary butyl alcohol. This suggests that all the components were stable for the 72 h of autosampler stability, confirming the previous finding.¹²

To demonstrate that the extracts of casework samples are stable for up to 72 h after extraction, a chromatographic analysis of the five casework samples was undertaken. The ANOVA of the PAR of one of the casework samples (37959902B) using F-test (single factor) gave $F_{\text{calc}} = 0.0429 < F_{\text{crit}} = 3.285$, demonstrating that there were no significant differences between the PAR over the 72 h. ANOVA of the pooled average response ratios gave $F_{\text{calc}} = 0.0429 < F_{\text{crit}} = 3.285$, demonstrating that there were no significant differences amongst the PARs for the samples belonging to the same group over the 72-h autosampler storage. From these data, it can be concluded that, as with simulated nyaope samples, casework samples of nyaope are stable up to 72 h after the preparation of drug extracts into tertiary butyl alcohol.

Hierarchical cluster analysis

HCA, using agglomerative clustering and unweighted linkage, was conducted on the average concentrations of caffeine, diamorphine, efavirenz, nevirapine, phenacetin and Δ^9 -THC for each of the 18 blind simulated and the 5 casework nyaope samples. The matrices generated for the HCA clustering indicated in Table 6 for the time interval t=72 h, demonstrate that the HCA method was suitable for discriminating the samples into different classes.²⁵ The matrices demonstrate that there was a maximum distance between Class 2 and Class 3 for the simulated samples and between Class 1 and Class 3 for the casework samples.

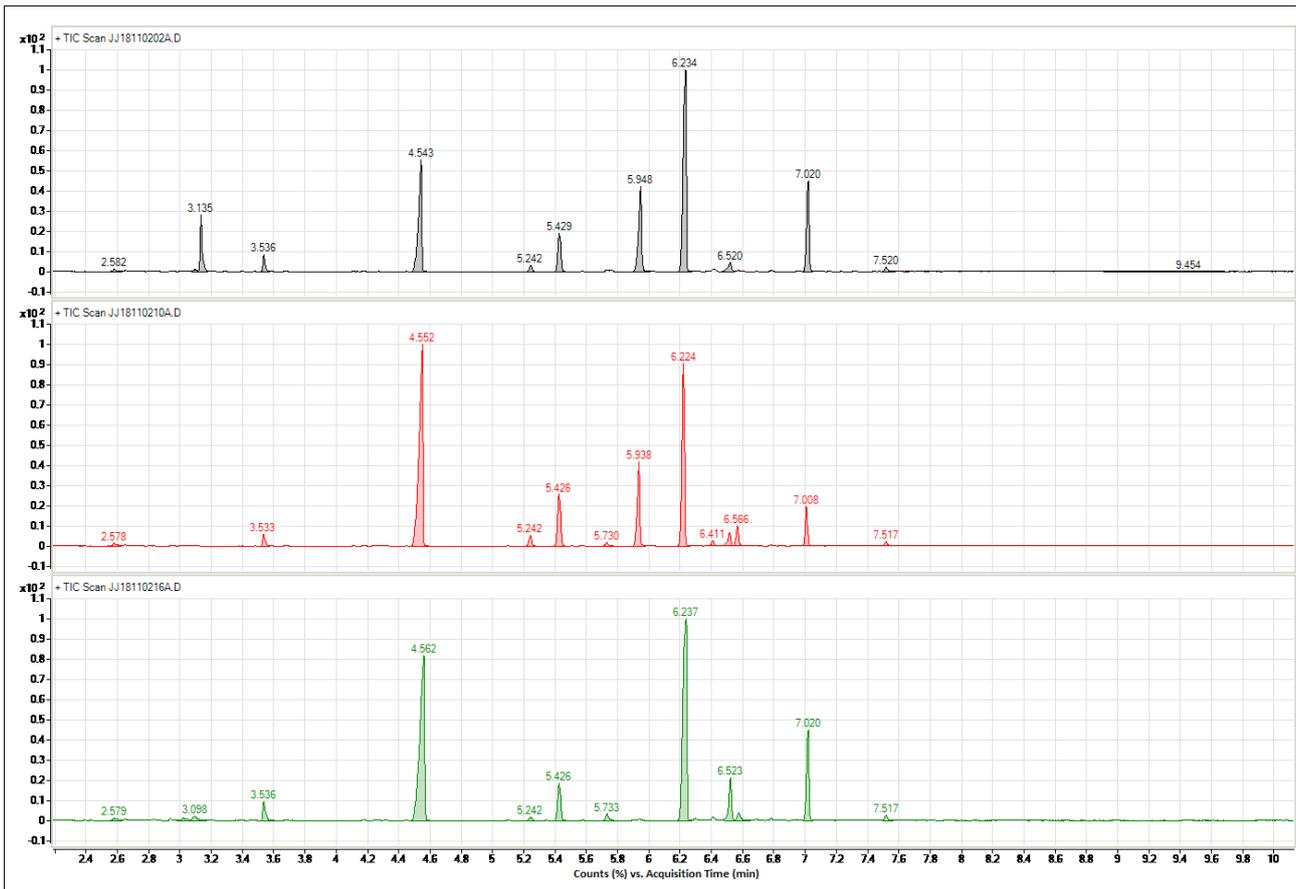


Figure 1: Exemplar chromatograms obtained by gas chromatography – mass spectrometry analysis of the three batches of simulated nyaope showing that it is possible to differentiate different batches of the drug, where identifications were (2.582) caryophyllene, (3.135) phenacetin, (3.536) caffeine, (4.543) efavirenz, (5.242) tetrahydrocannabivarin, (5.429) tetracosane IS, (5.733) cannabichromene, (5.948) nevirapine, (6.234) Δ^9 -THC, (6.411) cannabigerol, (6.520) cannabivarin, (7.020) diamorphine, (7.520) nonacosane, (9.454) unknown in the first chromatogram.

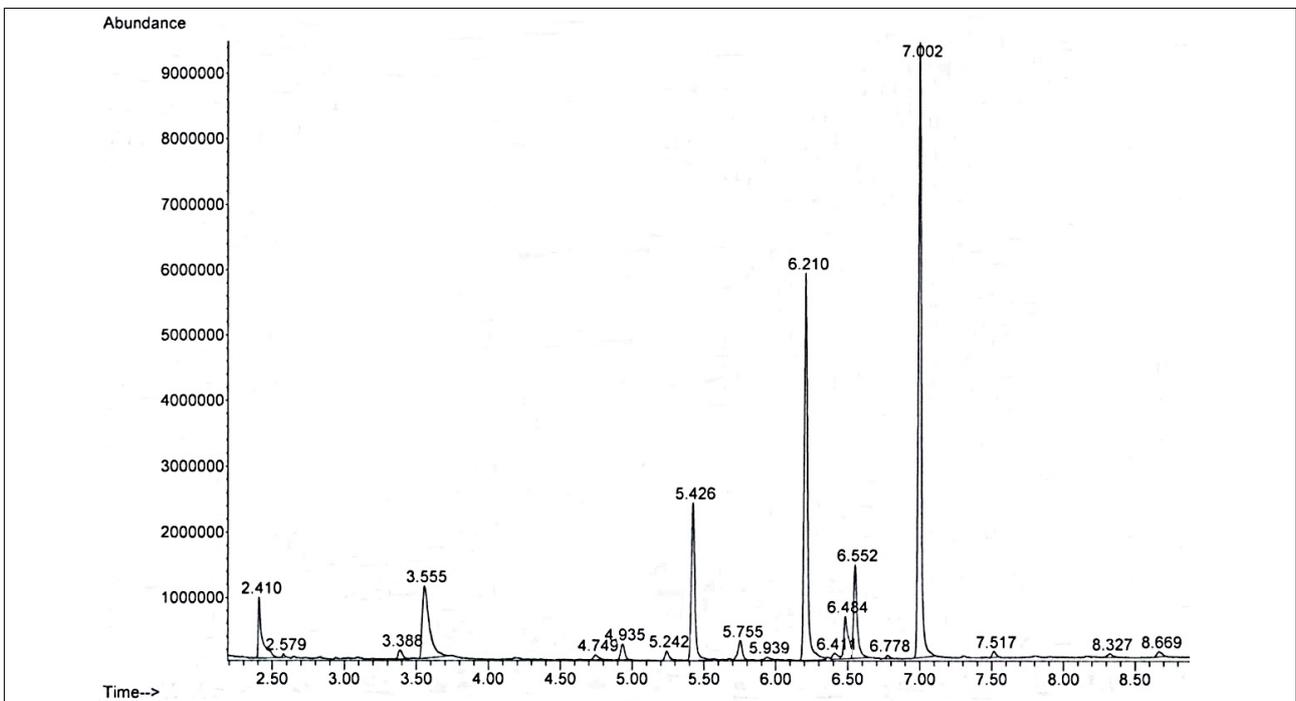


Figure 2: A typical total ion chromatogram for one of the casework samples (37959902B): (2.410) nicotine, (2.579) caryophyllene, (3.388) unknown, (3.555) caffeine, (4.749) methaqualone, (4.935) unknown, (5.242) tetrahydrocannabivarin, (5.426) tetracosane IS, (5.765) cannabivarin, (5.939) codeine, (6.210) Δ^9 -THC, (6.411) cannabigerol, (6.484) acetylcodeine, (6.552) 6-monoacetylmorphine, (7.002) diamorphine, (7.517) nonacosane, (8.327) unknown, (8.669) vitamin E.



Table 4: Components identified in the five casework samples

Sample component	2514202B	2520902B	3400002B	37959902B	50390902B
Bulnesol	×	√	×	×	×
Acetaminophen	√	×	×	×	×
Acetylcodeine	×	×	√	√	×
Caffeine	√	√	√	√	√
Cannabichromene	√	√	√	×	×
Cannabicomaronone	×	√	√	×	×
Cannabidiol	×	×	×	√	×
Cannabigerol	√	√	×	√	×
Cannabinol	√	√	×	×	×
Cannabivarin	×	√	×	×	×
Cocaine	×	×	√	×	×
Codeine	×	×	×	√	×
Diamorphine	√	×	√	√	√
Methaqualone	×	√	×	√	√
6-monoacetylmorphine	√	×	√	√	√
Nicotine	×	√	×	√	√
Nonacosane	√	√	×	√	×
Tetrahydrocannabivarin	√	√	√	√	×
Δ ⁹ -Tetrahydrocannabinol	√	√	√	√	√
Vitamin E	×	×	×	√	×

√ = identified
× = not identified

Table 5: Concentration of actual street nyaope samples (mg/mL x 1000)

Sample	Caffeine				%RSD	Diamorphine				%RSD	Δ ⁹ -Tetrahydrocannabinol				%RSD
	0 h	24 h	48 h	72 h		0 h	24 h	48 h	72 h		0 h	24 h	48 h	72 h	
2514202B	< QL	< QL	< QL	< QL	< QL	0.21	0.20	0.21	0.21	4.00	5.48	4.97	5.21	5.25	4.00
2520902B	< QL	< QL	< QL	< QL	< QL	nd	nd	nd	nd	nd	1.44	1.39	1.32	1.32	4.34
3400002B	0.50	0.50	0.49	0.49	1.47	2.53	2.42	2.44	2.38	2.52	0.32	0.33	0.31	0.30	3.92
37959902B	0.44	0.42	0.42	0.42	2.19	2.10	2.08	2.11	2.03	1.67	0.87	0.85	0.78	0.84	4.70
50390902B	0.82	0.80	0.81	0.81	0.94	2.87	2.82	2.82	2.79	1.10	0.11	0.10	0.10	0.10	6.36

<QL, below quantification limit; nd, not detected

Table 6: Matrices showing distances between central objects at t=72 h for the simulated nyaope samples S2, S3 and S18 and casework samples A2, B3 and C1

Blind simulated samples				Case work samples			
Class	1 (S2)	2 (S3)	3 (S18)	Class	1 (A2)	2 (B3)	3 (C1)
1 (S2)	0	2.92	2.34	1 (A2)	0	3.72	5.22
2 (S3)	2.92	0	3.63	2 (B3)	3.72	0	2.62
3 (S18)	2.34	3.63	0	3 (C1)	5.22	2.62	0

They further demonstrate that there was a minimum distance between Class 1 and Class 3 for the simulated samples and between Class 2 and Class 3 for the casework samples.

The results of the HCA performed on both the simulated and casework nyaope samples is also indicated in the dendrograms in Figure 3 for the time interval $t=72$ h. The results demonstrate that HCA successfully discriminated both the simulated and casework samples into three and five different batches, respectively. The HCA further demonstrated that both simulated and casework nyaope samples can still be discriminated even after 72 h of autosampler storage.

Principal component analysis

The correlation matrices for the PCA analysis of both simulated and casework nyaope samples (Table 7) indicate that all values are different from zero at a significance level of $\alpha=0.95$ (two-tailed), which indicates that there is a linear correlation between the variables.²⁶ The transpose of these correlation matrices are identical to the matrices themselves. As a result, their product would yield identity matrices, which demonstrates that the PCA is orthogonal.²⁶ The PCA indicates that there are three principal components (F1, F2 and F3) for the simulated samples and one principal component (F1) for the casework samples that explain the variability of the variables.

The eigenvalues of these principal components were greater than 1.00. The total variability (%) of the principal components (F1 and F2) for the simulated samples was more than the minimum 70%, while the principal component F1 alone accounted for more than 70% of the variability for the casework sample. This further indicates that the

two principal components, F1 and F2, for the simulated samples, and the single component F1 for the casework sample, are sufficient to explain the variability of the data set.^{27,28} As indicated in Figure 4, the PCA discriminated the simulated samples into three different batches and the casework samples into five different batches, similar to the observation made using the chromatographic profiles and HCA. The PCA further demonstrated that the simulated nyaope samples could still be discriminated even after 72 h of autosampler storage, confirming the stability of the samples once extracted into tertiary butyl alcohol.

Conclusions

We describe, for the first time, a method for the comparative analysis of nyaope. Provided that the samples are correctly seized and stored¹², extracted into tertiary butyl alcohol¹¹, and analysed within 72 hours¹¹, this study demonstrates, for the first time, that quantitative comparisons of nyaope samples can be made. It also demonstrates, for the first time, that clustering techniques can be successfully applied to nyaope samples to identify different members of the same batch. This means that law enforcement agencies in the Southern African Development Community and beyond have, for the first time, the ability to analyse nyaope and compare forensic science data. This will allow distribution and trafficking routes to be identified and will assist in the determination of the origins of this drug. It does, however, require that decisions are made about how the samples will be treated prior to any investigative activity. It has been shown¹² that the extraction of the drugs for analysis should be made as soon as possible after samples are seized, and this requires planning before any police action.

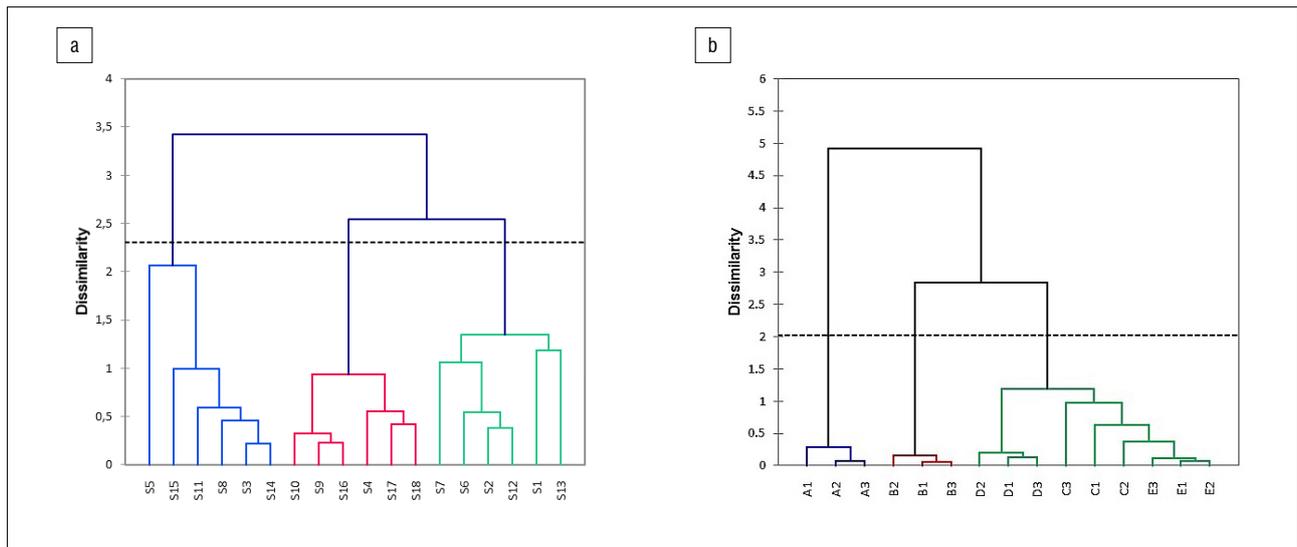


Figure 3: Dendrograms of (a) blind simulated nyaope samples and (b) casework samples analysed by hierarchical cluster analysis using unweighted linkage and Euclidean distance for the time interval $t=72$ h.

Table 7: Pearson correlation matrices for the simulated and casework samples

Simulated samples							Case work			
Variables	Caffeine	Diamorphine	Efavirenz	Nevirapine	Phenacetin	Δ^9 -THC	Variables	Caffeine	Diamorphine	Δ^9 -THC
Caffeine	1	0.884	0.595	-0.771	0.125	0.981	Caffeine	1	0.984	-0.710
Diamorphine	0.884	1	0.290	-0.471	0.556	0.858	Diamorphine	0.984	1	-0.687
Efavirenz	0.595	0.290	1	-0.645	-0.437	0.512	Δ^9 -THC	-0.710	-0.687	1
Nevirapine	-0.771	-0.471	-0.645	1	0.412	-0.801				
Phenacetin	0.125	0.556	-0.437	0.412	1	0.089				
Δ^9 -THC	0.981	0.858	0.512	-0.801	0.089	1				

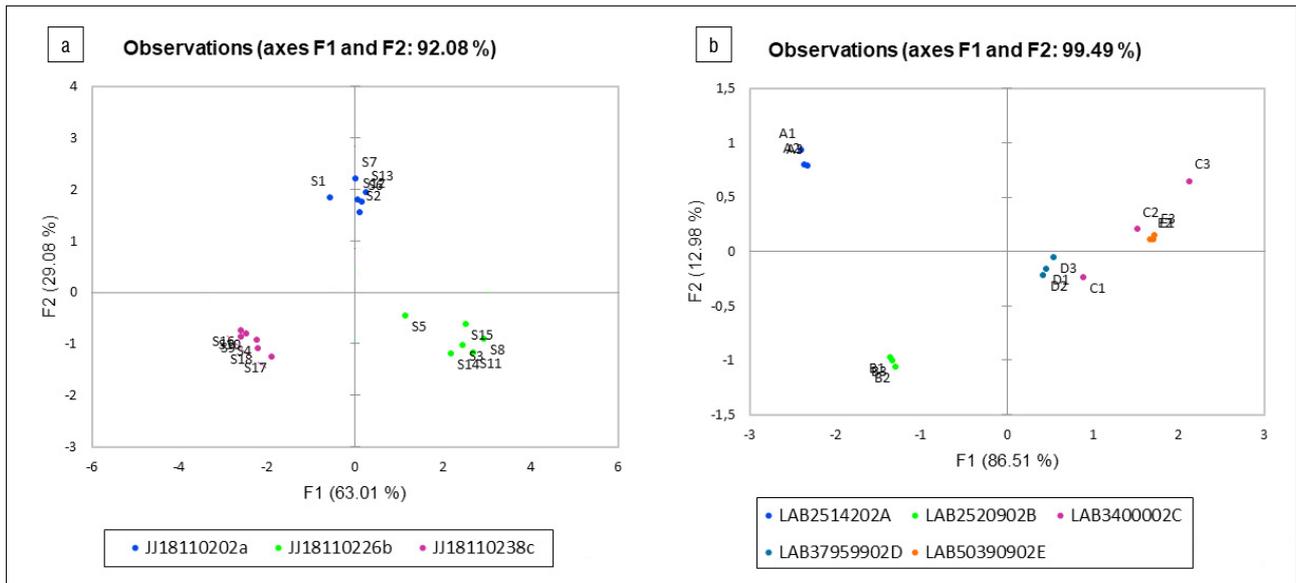


Figure 4: Observation axis for (a) blind simulated nyaope and (b) casework samples analysed using principal component analysis.

It is also interesting that whilst antiretrovirals have been reported to be present in nyaope, in the five casework samples analysed here, the antiretrovirals efavirenz and nevirapine were not identified. It may be that they were below the detection limit of the instrument or that they were indeed absent. However, the method does provide for the determination of these antiretrovirals where they are present at concentrations above the detection threshold. It should, however, be noted that other antiretrovirals cannot be detected by GC–MS, but that liquid chromatography – mass spectrometry (LC–MS) is a suitable method. However, LC–MS is not currently readily available to SAPS.

This method assists law enforcement and public health officials in a number of ways. It assists the law enforcement agencies in the identification and comparison of nyaope samples. It allows the establishment, for the first time, of a database on the composition of nyaope. It allows exchange of analytical data between jurisdictions, provided that the necessary quality control protocols are in place. It also facilitates the prosecution of trafficking offences. In terms of public health, it allows determination of the drugs present in nyaope and better public health information to be disseminated amongst the users of nyaope. In turn, they may choose, having this information, to avoid using this drug cocktail.

Acknowledgements

We thank members of the European Network of Forensic Science Institutes (ENFSI) Drugs Working Group, and especially Joana Alegre, Portuguese Criminal Police, for helpful discussion and their interest in this work. We thank the University of the Free State, South Africa, for financial support and the South African Police Service (SAPS) for support in conducting this research and for allowing the findings to be disseminated.

Competing interests

The research was supported by the South African Police Service Forensic Science Laboratory, of which P.M.M. is an employee. E.M.M. and M.D.C. have no conflicts of interest to declare.

Authors' contributions

P.M.M.: Conceptualisation, methodology, data collection, writing – original draft, review and editing. E.M.M.: Supervision, writing – original draft, validation, review and editing. M.D.C.: Supervision, writing – original draft, validation, review and editing.

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