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IDENTIFYING THE COMPOSITION OF STREET DRUG *NYAOPE* USING TWO DIFFERENT MASS SPECTROMETER METHODS

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ABSTRACT

Criminalization of trading and using of street drug *Nyaope* has had challenges in South Africa due to controversies about its composition. The high cost and complexity of its analysis using conventional chromatography methods also limit the testing availability in most routine laboratories. A state of the Art method with simple specimen processing and faster turnaround time at an affordable cost is urgently needed. To compare the ability of a new Time-of-Flight Mass Spectrometry with direct sample analysis (TOF-DSA MS) and Gas Chromatography Mass Spectrometry (GC-MS) methods in detecting the constituents of Nyaope against turnaround time and cost, in order to recommend a better system for routine use. Cross-sectional, qualitative and descriptive pilot study on samples purchased from various sources of 12 townships in Northern Gauteng Province. The constituents consistently detected in all samples were caffeine, drugs of abuse such as opiates, codeine, morphine, methyl-dioxy amphetamine (MDA) and heroin. Some samples contained antibiotics (citroflex) and antiretroviral drugs (zidovudine). Central nervous system (CNS) depressants such as phenobarbitone and benzodiazepines, benzitramide, moramide intermediates and thiofentanyl and stimulants such as Pipradol, and fenethyline were detected by the TOF-MS system. The usefulness of TOF-DSA MS was better as a screening method while GC-MS provides specificity and confirmatory detection. Due to direct sample analysis, the TOF-DSA provides analytical runtime of 15 sec while GC-MS takes 10 minutes per sample. The running cost for the GCMS is more expensive due to the high cost of reference materials and the need to perform specimen preparation as opposed to TOF-MS. We recommend TOF-DSA MS for initial screening of organic compounds in the Nyaope mixtures followed by confirmation by GC-MS for medico-legal interventions.

Key words: *Nyaope*, drug of abuse, Mass Spectrometry, Gas Chromatography, Anti-retrovirals

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INTRODUCTION

Nyaope, also known as *whoonga*, is a dangerous and highly addictive South African street drug (Department of Justice and Consitution SA, 2014). Nyaope is a fine brown powder due to its mixture with soil, sand or in some cases cement powder in order to disguise the underlying white power of drug of abuse. It is usually wrapped in marijuana (dagga) leaves and smoked. It is not always clear what all of the ingredients of Nyaope are, and the ingredients may vary from sources of sellers. But one thing is clear: Nyaope is very addictive and there are assumptions that it may include heroin, detergent powder, rat poison, and crushed anti-retroviral drugs (ARVs) (Facts for Nyaope, 2014). ARVs are the medications used to treat patients with HIV. In most users, Nyaope not only gives strong addiction but also severe abdominal pain and seizures which are withdrawal symptoms. With respect to drug to drug interaction between various drugs of abuse, clinical studies showed that they aggravate the body's addictive reaction and also causes untoward effects such as acute abdominal pain, seizures, vomiting, salivation, psychosis etc (Bruce, Altice, & Friedland, 2008). This makes the user consume more and more Nyaope due to the belief that consuming more would relieve these symptoms. Nyaope is relatively cheap - about R20 to 30 for one "hit" and a person can become highly addicted after using the drug only once (Department of Justice and Consitution SA, 2014.). A user will soon feel as if he needs several hits to make it through the day. Until and unless the exact nature of the contents of this street drug is understood, treatment strategies and rehabilitation support for the users may be difficult to achieve.

Figure 1. Nyaope sample

Nyaope first appeared in the townships around Durban in 2010, but is reportedly moving to impoverished areas around South Africa. Nyaope is a serious threat to South Africa's HIV-positive population. There have been reports of gangs robbing HIV/AIDS clinics in Soweto to obtain ARVs for making Nyaope, as well as addicted users mugging ARV patients to obtain the drugs for themselves. It also promotes drug resistance to ARVs which has a huge impact on the treatment programs for patients living with HIV/AIDS ("Facts for Nyaope," 2014). Since Nyaope appears similar to soil or cement powder, it is not easy to apprehend the users, sellers or distributors. Analysis of Nyaope is not done routinely at medical laboratories or forensic laboratories due to the high cost and complexity of conventional chromatography methods and lack of experience in dealing with materials of such nature.

The newly introduced TOF-MS method uses direct sample analysis and recent studies showing its ability in identifying individual compounds in the simulated mixture of drugs encourages the researchers to test its ability in the real drug mixture samples (Wilhide, Lacourse, & Crowe, 2013)

The objectives of this study were to identify the exact constituents of Nyaope by comparing the performance of two

analytical methods, namely the conventional GC-MS (Gas Chromatography Mass Spectrometry) and the new TOF-MS (Time of Flight Mass Spectrometry).

MATERIAL AND METHODS

Ethical approval for this study was obtained from the Medunsa Research Ethics Committee (MREC/H/165/2012:IR). The research method applied was a cross-sectional descriptive and comparative study.

Sample

Nyaope samples were randomly collected through the users from the townships and urban areas surrounding Pretoria in North Gauteng. The samples were collected over a period of one week from 12 areas in Gauteng province (with the number of samples collected from each area shown in brackets); Pretoria central (5), Sunnyside (4), Mamelodi (10), Bronkhorstspruit (3), Delmas (2), Winterveld (2), Ramogodu (3), Springs (1), Tembisa (4), Garankuwa (2), Soshanguve (2) and Witbank (2). However, obtaining Nyaope samples was a challenge as it requires building a relationship with the current users in order to build their trust and obtain their assistance.

Samples were collected in the sterile 30 ml specimen jars and were labelled with the name of the area and the number. Each specimen was thoroughly mixed before dividing it into two containers. The first container was marked A and the second one B. The lids were screwed tightly to prevent any leakage or contamination. All samples labelled A were analysed at the GC-MS laboratory at the SAPS forensic toxicology and the specimens labelled B were analysed at the Perkin Elmer Research and Development Laboratory in Midrand. All specimens were kept at room temperature and cool dry place during transportation to the laboratories.

Sample preparation before analysis

Gas Chromatography coupled Mass Spectrometry (GC-MS) is an internationally accepted analytical comparative technique where compounds are separated in the gas phase and a characteristic pattern (mass spectrum) of the separate compounds is obtained. Sample to be analysed, in a liquid form is injected into an inert gas stream (helium/argon) and swept into a column packed with a stationary phase inside the oven. Absorptive interaction between the components in the gas stream and the coating leads to a differential separation of the components of the mixture, which are then swept to the detector which is a Mass Spectrometry. Mass Spectrometry takes injected material, ionizes it in a high vacuum, propels and focuses these ions and their fragmentation products through a magnetic mass analyser and then collects and measures the amounts of each selected ion in a detector. When the interested compounds are not volatile in gas, the process of sample derivitization has to be done which is time consuming and the need to purchase the reference material for each compound in the mixture makes the testing very expensive (Foltz, Fentiman, & Foltz, 1980)

In the TOF-MS method, a PerkinElmer FlexarTMFX-15 LC pump with AxION™ TOF MS was used for analysis. It uses direct sample analysis with no need for sample preparation except extraction in liquid Methanol for the organic compounds. The supernatant was analysed through Time of Flight instrument by di-

rect application on the specimen grid. The extracted specimens are carried through the TOF path by the Nitrogen gas flow in a fixed temperature and gas pressure environment. The calibrator, Acetonitrile liquid was used for checking the resolution of this compound in an expected position and gamma hydroxyl butyric acid was used as a blank to check the baseline of separation and peak areas (Daugherty & Crowe, 2014)

Molecular identification of each compound in the mixture is achieved by its mass/charge (m/z) ratio which is matched to the best fit m/z in the isotopic patterns of known reference compounds prestored in the instrument software library. The sensitivity of the analysis is M/Z 100-1000 and error was < 1 ppm (parts per million). The direct sample application part can be removed and replaced by HPLC (High Performance Liquid Chromatography) for separation of compounds such as drug of abuse in more complex biological fluids (blood or urine) (Daugherty, 2011)

RESULTS

Both GC-MS and TOF-MS could report the compounds qualitatively although areas under the peaks may be used to estimate the amount of compound present. Table 1 represents the qualitative detection of the various compounds found in the Nyaope samples that were analysed. Although multiple samples were collected from most areas, representation of only one sample was chosen based on the highest number of compounds present.

Samples of Tembisa and Winterveld were not sufficient to share for GC-MS laboratory hence we could not report the spectra of drugs for these two areas done by GC-MS although the analyses of these samples were done on the TOF-MS.

Figure 4 represents the mass spectra of both methods for the specimen collected in Mamelodi Township as an example. In TOF-MS, calibrator's position is checked and correlate with its isotopic pattern and molecular mass. Then other organic compounds including drugs of abuse are identified in the same way.

DISCUSSION

GC-MS method could report the compounds quantitatively if the reference materials or internal standards are used for calibration of concentrations against m/z ratios of ionized molecules. However

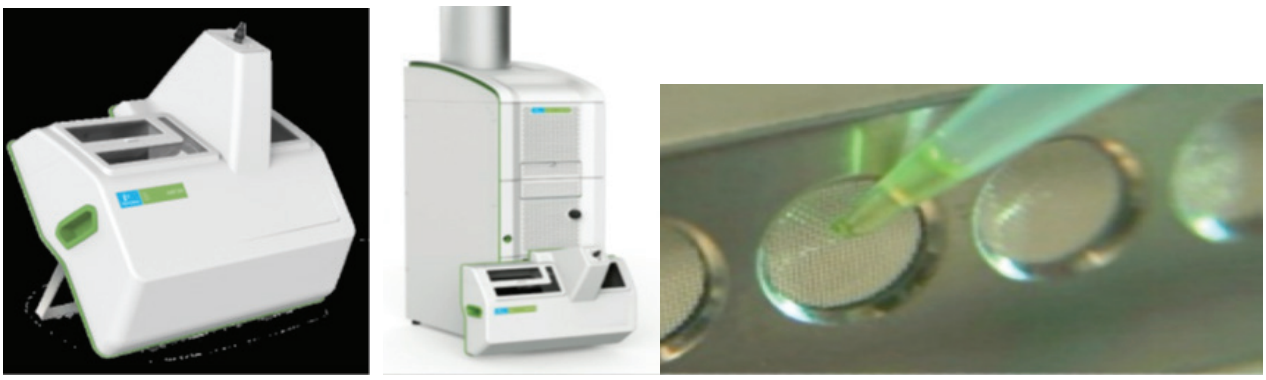
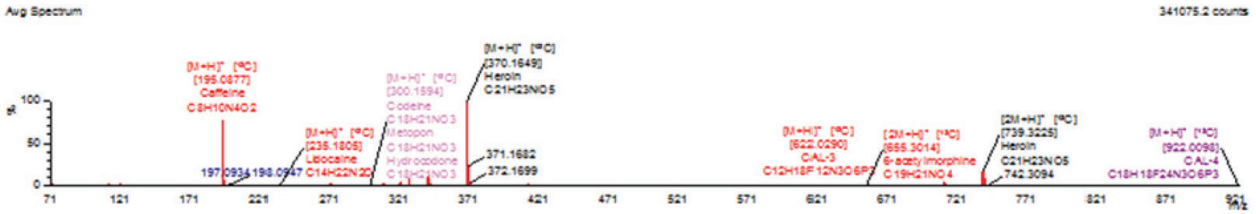


Figure 2. From left to right, direct sampling port, Time of Flight piece of the instrument TOF-MS and picture of sample application on to the disposable wire mash.

Table 1. Constituents of Nyaope samples acquired from various townships analysed by two different mass spectrometers

		CONSTITUENTS IDENTIFIED IN NYAOPE																	
Area and number of sources	Method	Caffeine	Acetaminophen	Opiate (meconin, methadone.papaverine, Dimenoxitol	Dextromethophan	Codeine/metabolites	Morphine /metabolites	Heroine	Amphetamine / meth- metabolites	Cathine (b OH amphet)	Citroflex A	Duracaine/lidocaine	Anti-retroviral (zidovudine)	Thiofentanyl	Benzitramide (narcotics)	Benzodiazepines	Phenobarbitone	Pipradol (Dopamine reupt)	Moramide narcotics	Fenethyline (stimulant)
Garankuwa (sample 2)	GC	+	+	+	+	+	+	+												
	TOF	+	+	+	+		+	+	+											
Soshanguve (sample1)	GC	+				+	+	+				+								
	TOF	+	+				+	+	+											
Bronkhorstspruit (sample 2)	GC	+				+	+	+			+									
	TOF	+		+			+	+	+	+					+		+	+	+	
Witbank (sample 2)	GC	+	+		+	+	+	+	+											
	TOF	+	+		+			+	+											
Mamelodi (sample 8)	GC	+			+		+	+	+											
	TOF	+		+	+	+	+	+	+	+		+		+						
Springs (sample 1)	GC	+	+		+	+	+	+												
	TOF	+		+	+			+	+						+					
Pretoria central (sample 5)	GC	+	+		+	+ +	+	+												
	TOF	+	+	+	+			+	+				+			+				
Ramogodu (sample 3)	GC	+						+												
	TOF	+	+				+	+	+	+			+							
Winterveld (sample 2)	GC	Not done due to insufficient samples																		
	TOF	+		+			+	+	+	+					+					
Delmas (sample 2)	GC	+			+	+	+	+												
	TOF	+			+		+	+	+	+										+
Tembisa (sample 4)	GC	Not done due to insufficient samples																		
	TOF	+		+		+		+	+					+	+			+	+	
Sunnyside (sample 9)	GC	+	+			+	+	+												
	TOF	+					+	+	+	+			+	+	+	+				

TOF-MS: average spectrum



GC-MS:

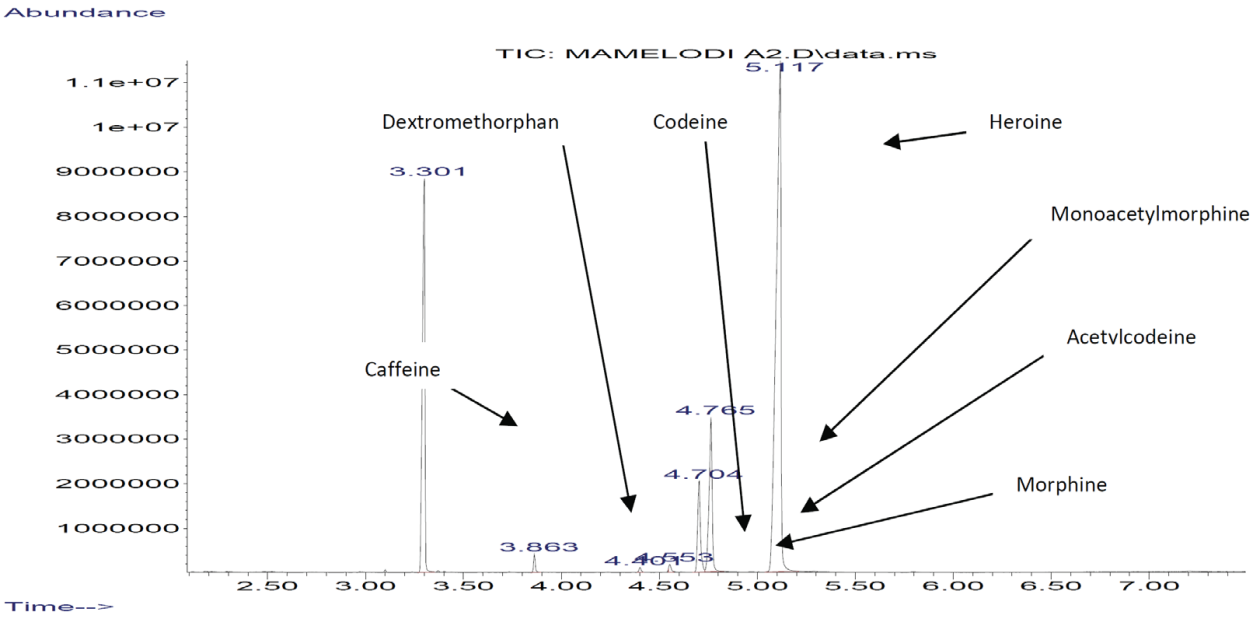


Figure 3. Example of Mass Spectra for one selected Nyaope specimen collected in Mamelodi township of Pretoria

using isotopic internal standards are relatively more stable than the natural compound but they are more costly (Karacic & Skender, 2000). Another study showed analysis of drugs of abuse in hair by LC-MS method (liquid chromatography) and it also uses expansive reference compounds, accessories and the method is complex requiring highly skilled operators (Paterson, Mclachlan-troup, Cordero, Dohnal, & Carman, 2001). In our study, to compare the performance of the two methods, we reported results qualitatively from both GC-MS and TOF-MS systems.

Consistently seen in all samples were caffeine, drugs of abuse such as opiates, codeine, morphine, methyl-dioxy amphetamine (MDA) and Heroine. A local anaesthetic Duracaine/lidocaine was also found in some samples which ~~It~~ has a mood enhancing effect. Detected by the TOF-MS but not by GC-MS were the antibiotic (citroflex) and the ARV zidovudine, CNS depressants such as phenobarbitone and benzodiazepines, benzitramide, moramide intermediates and thiofentanyl, stimulants such as Pipradol, and fenethyline. This could be due to the fact that GC-MS system requires reference materials of each of these compounds to verify and discriminate them from other compounds. Of note, Dextromethorphan, the antitussive cough suppressant was detected by both systems.

Resolution of unidentifiable peaks is challenging for both methods. In TOF-MS, the unknown peak can be matched in the instrument software library or on internet based on correlation of molecular mass and isotonic pattern and aim for more than 95% of match. In order to facilitate the accuracy of identification, drugs of abuse artificially made up in a mixture (without any soil, sand or cement like in Nyaope) was analyzed on TOF-MS by Perkin Elmer to identify and save the isotopic patterns in the instrument library (Wilhide et al., 2013) (Robinson, 2010). This makes the system possible to match 100% fitness with the patterns received from analysis of Nyaope samples. Confirmation of identified peaks is much efficient with GC-MS system as it can fragment the peak into various ions which show a specific pattern of mass and charge ratio however a reference material is needed to discriminate this pattern from the closely related other compounds for confirmation(Zhao & Zhang, 2012)

Regarding the analytical runtime, the TOF-MS produces results in 15 seconds for a mixture as opposed to GC-MS which takes 10 minutes for each compound. Time form collection to reporting of results marks the turnaround time and it may depend on the workload and specimen batching requirement of the testing laboratory for cost-effectiveness on the reagents and reference materials especially for the GC-MS lab.

Cost-wise, GC-MS is more expensive due to high cost of reference material and the need to perform specimen derivitisation for certain compounds. It costs approximately R 560 for quantitative drug assay per sample according to the scale of benefit guideline for South Africa. However, the discriminatory power of GC-MS due to its higher specificity to detect a particular suspected compound/drug in a sample and also ability to report in quantitative level provides a platform for confirmation of presence of a drug of abuse which is required for medico-legal procedures. Comparatively, the consumable cost for TOF-MS is R 10 for a disposable sample grid. Each specimen uses 500 micro litre of Methanol for extraction before analysis. With this relatively inexpensive running cost and faster analytical time, TOF- DSA MS provides an opportunity for screening of drugs and compounds in a mixture of unknown whereas the GC-MS system is more useful in confirmation of a particular screened positive compound in the same mixture.

Contrary to prior assumptions, our samples did not show any inclusion of rat poison but the anti-retroviral drug was found to be present in few samples of two areas. This has shed the light on perceptions and beliefs of users who are also living with HIV/AIDS in these areas and townships in their adherence problems due to poor tolerance to the side effects of ARV therapy. Consumption of ARVs in such inhalational form may enhance drug resistance or damage to the respiratory mucosa although no study has been done to prove this. This important finding has an impact on the HIV treatment programs nationwide and has to be taken into consideration when counselling for adherence is provided for patients living with HIV.

CONCLUSION

Identifying the constituents of Nyaope is important in order to select appropriate antidotes for the treatment of with-

drawal symptoms during rehabilitation as well as providing evidence for criminalization of dealers and users. The challenge is the heterogeneity and constant change in its formula depending on the availability of constituents in the community. For unknown drug mixtures such as Nyaope, TOF- DSA MS provides a better platform for the initial screening of constituents while GC-MS provides a confirmatory testing of a specific compound indicated as screen positive in the TOF-MS.

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REFERENCES

Bruce, R. D., Altice, F. L., & Friedland, G. H. (2008). Pharmacokinetic drug interactions between drugs of abuse and antiretroviral medications: implications and management for clinical practice. *Expert Review in Clinical Pharmacology*, 1(1), 115–128.

Daugherty, S. (2011). *Direct sample analysis using Time of Flight Mass Spectrometry*. PerkinElmer Report.

Daugherty, S., & Crowe, H. (2014). *Automated Direct Sample Analysis (DSA / TOF) for the Rapid Testing of Drug Compounds*. Perkin Elmer Report

Department of Justice and Consitution, S. A. (2014). *THE END FOR NYAOPE! News Letter*, Retrieved Aug 24, 2014 from http://www.justice.gov.za

Facts for Nyaope. (2014). *HIV South Africa, mobilization*. Retrieved Aug 25, 2014 from http://hivsa.mobi/h4l/art.php?art=147

Foltz, R. L., Fentiman, A. F., & Foltz, R. B. (1980). *GC / MS assays for abused drugs in Body Fluids.* Monograph, National Institute for Drug Abuse, USA.

Karacic, V., & Skender, L. (2000). Analysis of Drugs of Abuse in urine by Gas Chromatography/Mass Spectrometry: Experience and Application. *Archive in Radiation and Toxicology,* 2000;51:389–400, 51, 389–400.

Paterson, S., Mclachlan-troup, N., Cordero, R., Dohnal, M., & Carman, S. (2001). Qualitative Screening for Drugs of Abuse in Hair Using GC-MS. *Journal of Analytical Toxicology*, *25*(April), 203–208.

Robinson, K. (2010). AxION DSA: *Quality data without chromatography or sample preparation*. Perkin Elmer Report

Wilhide, J., Lacourse, W. R., & Crowe, H. (2013). *Confirmation of drugs of abuse in pseudo drug mixes using HPLC- ESI TOF MS*. Poster, American Association of Clinical Chemistry annual congress.

Zhao, S., & Zhang, R. (2012). *A Rapid Method for Detection of Drugs of Abuse in Blood Samples Using the Thermal Separation Probe and the 5975T LTM GC / MS*. Application Note.Institute of Forensic Science, Shanghai, China.