The qNMR Analyses of Nicotine in Vape Liquids Sold in South Africa

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ABSTRACT

The use of e-cigarettes has become widespread in South Africa, with hundreds of brands being marketed and an even greater selection of "vape liquids" available. Vape liquids usually contain a mixture of propylene glycol (PG) and vegetable glycerin (VG), nicotine, flavourings, and various other additives. In South Africa, and in fact globally, there is little regulation around the sale and production of vape liquids. Some recent publications have reported on the potential dangers associated with the lack of regulation, citing incorrect and dangerously high nicotine levels and the confirmed presence of nicotine in "nicotine free" labelled vape liquids. In this study, 20 samples of vape liquids were sourced from local retailers and informal traders and were analysed using NMR spectroscopy. The concentrations determined by NMR were generally not in agreement with the concentrations indicated on product labels. It was found that the products generally contained between 2-33% of the indicated nicotine concentration and, that the PG:VG ratio was significantly different from what the label indicated. Only 1 of 7 South African vape liquid samples which were labelled as "nicotine free" contained nicotine. Ethylene glycol (EG) and related polyols were not detected in any of the 20 samples tested. These findings demonstrate significant inconsistencies in vape liquid composition and labelling.

KEYWORDS

Vape liquids, PG, VG, NMR spectroscopy, nicotine

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INTRODUCTION

The number of global e-cigarette users have grown exponentially over the past 10 years. Some recent analytical reports suggest that e-cigarette liquid contents are not correctly indicated or clearly displayed on the package labelling.[1,2] The incorrect labelling, intentional or otherwise, may have a direct impact on public health and, the extent of the inaccuracy and mislabelling is particularly concerning with regards to youth uptake and addiction. There are, to date, no publications which have evaluated the contents of vape liquids used in e-cigarettes in South Africa. The e-cigarette, first introduced in 2003 as a tobacco cigarette alternative, has rapidly been gaining popularity in South Africa, particularly among South African youth. A recent publication reported that nearly 50% off stores selling e-cigarettes and related merchandise were generally situated within 5km of higher education institutes.[3] The increase in e-cigarette popularity has also been accompanied by an increase in the number of different vendors and vendor websites. In 2016, Caruana reported that there were around 60-70 franchises in the country, with one of the biggest e-cigarette brands at the time reporting a 4000% growth that same year.[4] The trend is expected to have continued over the past few years with Euromonitor having forecasted a 47.7% growth for e-cigarettes in South Africa between 2016 and 2021.[5] As these trends will likely continue, it is important to investigate the quality and regulatory control space around the vape liquid and e-cigarette industry, as well as the related health implications, if any, of a growing e-cigarette user population. In South Africa, there are currently no reports of analytical testing having been carried out on vape liquids. This study therefore explores the composition of some of the vape liquids available to South African consumers. Some of these vape liquids are known to be produced locally without any apparent guiding regulatory policy or testing, where the onus is on the vendor to ensure that certain quality standards are being met (e.g ISO 7).[6] This investigation therefore aimed to identify levels of incorrect and inaccurate labelling, with a focus on nicotine and other potentially harmful chemical substances such as ethylene glycol. Generally, vape liquid suppliers will not disclose detailed ingredient information on packaging and will report only on the excipient mix and the concentration or absence of nicotine.

Reports on the composition of vape liquids, on the international market, using high pressure liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC–MS) have indicated significant variability in product composition.[2,7,8] One study found that 6 out of 10 vape liquids which were branded as "nicotine free" contained nicotine, with nicotine concentrations between 1-3 mg/mL.[9] Some reports have also highlighted concerns around the presence of illicit and psychoactive drugs which are not declared as part of the ingredients.[10-12] One report found that 5 of 28 vape liquid samples analysed contained ethylene glycol instead of PG and/or VG as the major component.[13] Ethylene glycol (EG), unlike PG and VG, is not a GRAS (generally recognized as safe) chemical and is considered a greater toxicological hazard. These reports demonstrate the need for more widespread testing of vape liquids which may, in turn, strengthen the call for better regulation and quality control.

Vape liquid products produced locally are generally prepared from food or pharmaceutical grade PG and VG. Which are combined in various ratios, typically either 30:70 or 50:50. The PG/VG mixture is then used as the base for the vape liquid to which nicotine and flavourings are added to afford different vape liquid products. Many vape liquid producers will offer the same product at varying concentrations of nicotine, commonly prepared concentrations include; 3, 6, 9, 8, 12, 16 and 35 mg/mL or nicotine free. It is worth noting that the varying concentrations of nicotine available in vape liquid products may all afford comparable nicotine delivery based on the resultant nicotine concentration in blood.[14] A recent publication also indicated that nicotine in the aerosol form may not be well correlated to the varying nicotine concentrations in the vape liquid.[15] However, consumers should be aware if they are being exposed to nicotine and products containing nicotine and related compounds should be clearly labelled, as legally required. Nicotine concentrates, which contains either nicotine free base or nicotine salts dissolved in PG or VG, are generally used to produce vape liquids. Since All of the required components, including flavouring compounds, are available for online purchase, it has become relatively easy to prepare

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and sell home-made vape liquid. These vape liquids are then generally not subjected to any testing and some traders will sell vape liquids produced in this way as third-party items.

The general analytical techniques employed in determining vape liquid composition include gas chromatography (GC), GC-MS, liquid chromatography-mass spectrometry (LC-MS) and nuclear magnetic resonance (NMR) spectroscopy.[16,17] GC and LC-MS related techniques often require extractions; NMR spectrometry however allows for the direct measurement of the material and provides both qualitative and quantitative data.[18] A recent publication where ^{1}H NMR was used to quantify nicotine, PG, VG and various other chemicals commonly reported in vape liquids, reported that the concentrations determined by NMR were comparable to that of HPLC and GC-MS.[19] Similarly, this method of quantitative NMR (qNMR) measurement was also used to quantify the levels of CBD in South African consumer goods while another recent publication utilised qNMR coupled with progress curve analysis to track enzyme kinetics.[20,21] To date, there are limited reports on the use of NMR spectroscopy for the analysis of vape liquids. Additional studies are therefore needed to expand on what is known about the local vape liquid market and, such investigation will further probe whether NMR spectroscopy could be used as a rapid method for quantifying common vape liquid components.

Given the widespread use and growing popularity of e-cigarettes, developing and refining analytical techniques that can be used to rapidly evaluate products on South African market have become increasingly important. Here we explore the use ^{1}H NMR spectroscopy to characterize and investigate the composition of 10 vape liquid samples, with a focus on the PG/VG ratios and nicotine levels across the various products. The concentrations of these components were also compared to what was reported on their respective product labels.

EXPERIMENTAL

Sampling

The vape liquid samples analysed in this study were selected to reflect products that are easily accessible to consumers in the South African market. Sampling focused on commonly available brands that could be readily purchased through local vape retail outlets and online vendors operating within South Africa. Vendors were identified through consultation with retail stores focusing on products frequently stocked or advertised to consumers. Products were then selected to include a range of commonly encountered nicotine strengths as well as items marketed as "nicotine free". Preference was given to brands that appeared frequently across multiple vendors, as these were considered representative of products most likely to be encountered by typical consumers. Vape liquid samples (n = 20) were then analysed which included vape liquids containing nicotine (n = 13) and those marketed as nicotine free (n = 7). Products identified as popular and readily available to local consumers were chosen. This was done through consultation with retail outlets and vendors. All the vendors were located in South Africa and only one of the vendors declared that they produced and sold their own vape liquid, the others only sold and did not produce their own products. It should be noted that not all samples were confirmed to be locally produced as some samples had packaging that indicated otherwise, despite being advertised as locally produced. Samples that were purchased online were chosen from among brands which claimed that all their products were produced locally. For the nicotine containing samples, products with varying nicotine content were obtained, as indicated by the label.

NMR analyses

The NMR analyses of vape liquid samples were carried out using adapted procedures previously reported for the analysis of tobacco liqueur and vape liquids.[19,22] The vape liquid samples were dissolved in a NMR buffer containing an internal standard, prior to analysis.

Preparation of vape liquid NMR samples: 60 µL of each sample vape liquid sample was mixed with 480 µL of water (distilled) and 60 µL of a D_2O-based NMR buffer containing a 10 mM internal standard (pH 7.4; 1.5 M KH_2PO_4, 10 mM 3-(trimethylsilyl)-propionate acid-d_4 (TSP), 3 mM sodium azide). The resulting solutions were briefly mixed using a vortex mixer and, thereafter, 500 µL of each sample was transferred to a 5 mm NMR tube for qNMR acquisition.

Preparation of standard solutions: All materials were purchased from Merck Life Science (Pty) Ltd. Standard solutions were prepared from a 10 g/L stock solution (*S*-nicotine bitartrate, propylene glycol, ethylene glycol, 1,3-butanediol, 1,3-propanediol, diethylene glycol and glycerol, all dissolved in distilled water). Standard solutions of each of the analytes were prepared by diluting the stock solutions in distilled water and NMR buffer. The standard solutions were prepared in triplicate at concentrations of 1000, 500, 100, 50, 10, 5 and 0.1 mg/mL for the polyols, while nicotine standards were prepared at concentrations of 50, 10, 5, 1, 0.5, 0.1, 0.05 and 0.01 mg/mL. The resulting solutions were briefly mixed using a vortex mixer and, thereafter, 500 µL of each sample was transferred to a 5 mm NMR tube for qNMR acquisition. Calibration curves were prepared by comparing the integration of compound specific resonances against the normalised integration of the internal standard, TSP.

Preparation of standard vape samples: Standard solutions of vape samples were prepared by dissolving nicotine in VG only to afford nicotine concentrations at 35, 18 and 8 mg/mL. The standard solutions were prepared in triplicate at each concentration and 60 µL of each of the vape liquid samples were mixed with 480 µL of distilled water and 60 µL of NMR buffer. The resulting solutions were then transferred to a 5 mm NMR tube for qNMR acquisition.

All NMR measurements were performed on a Bruker 400 Ultrashield Plus spectrometer (Bruker Biospin, Rheinstetten, Germany) equipped with a 5 mm BBO probe, using a Bruker Automatic Sample Changer (SampleXpress lite) and all spectra were acquired at 300.0 K. The calibrated 90^O pulse (P1) for nicotine was determined using signals at 8.69 (1H, d, J = 1.7 Hz), 8.66 (1H, dd, J = 4.2, 1.2 Hz), 8.09 (1H, d, J = 8.0 Hz) and 7.62 (1H, dd, J = 7.9, 5.9 Hz) ppm, using the standard zg experiment. Using the calibrated P1, the longitudinal relaxation constant (T1) was estimated for both nicotine and the IS, using the standard Bruker Inversion-Recovery experiment (t1ir1d). The measured T1 was <2 s for both nicotine and the IS, resulting in selected relaxation delay (D1) of 10 sec. The NMR spectra of the aqueous solutions were acquired using a Bruker standard water suppression 1D zgpr pulse sequence with 64 scans (NS) and 4 prior dummy scans (DS). The delay (D1) used was 10 sec. The sweep width (SW) was 19.9914 ppm and the time domain of the FID was 65536 (65 k). The spectra of the vape liquid samples were compared to the spectra of the standard solutions. To quantify compounds of interest, separate peaks corresponding to each substance were identified and integrated against TSP as an intensity reference, using the NMRQ function on Topspin V3.2 (Bruker Biospin, Rheinstetten, Germany).

HPLC analyses

HPLC was recorded for the nicotine standard samples to confirm the purity of the commercially sourced material. HPLC analysis was performed on an Agilent 1260 Infinity II (Agilent Technologies, Santa Clara, CA) Series LC system equipped with a degasser, quaternary pump, autosampler, thermos column compartment and a variable wavelength detector (VWD). Sample data was acquired and processed using Agilent OpenLAB software.

Preparation of samples for HPLC analyses: 10 µL of the sample was added to 980 µL of methanol and 10 µL of 1.053 mg/mL ibuprofen (IBU) as the internal standard. The solution was filtered using a 0.22 µL nylon filter and transferred into an HPLC vial for analysis. HPLC analysis was performed on an Infinity Lab Poroshell 120 ECC18 column (4.6 x 150 mm) heated at 25 ^{o}C. The aqueous and organic mobile phases used

were H_2O and ACN. The autosampler injected 30 µL aliquots at a flow rate of 1 mL/min at an initial gradient of 95% H_2O to 5% ACN to reach 5% H_2O to 95% ACN over 3 min, with a 4 min hold of 95% ACN over 4min and finally, returning to 95% aqueous mobile phase over 10 min. The VWD detector was set to 230 nm with a signal peak width of > 0.0063 min (80 Hz).

Preparation of standard solutions: Working calibration standards were prepared by serial dilutions of a stock solution containing 5 mg/mL of nicotine in ACN to produce concentrations of 5, 2.5, 1.25, and 0.625 mg/mL respectively.

RESULTS AND DISCUSSION

As this study represents the first analytical investigation of vape liquid composition in the South African market, the sample set was selected to provide an initial exploratory snapshot of products readily available to consumers through both retail outlets and informal vendors. While the number of samples analysed is limited, it captures a range of commonly available brands and nicotine strengths, allowing for a preliminary assessment of labelling accuracy and product composition in a largely unregulated market. The ^{1}H NMR spectrum of a typical nicotine containing vape liquid sample in D_2O is shown in Figure 1. The largest signals in the spectrum are due to the presence of PG and VG which constitute the excipient mixture and are the major components of the vape liquid sample. The expanded portion of the ^{1}H NMR spectrum in Figure 1 (7 – 9 ppm) show the expected aromatic nicotine resonances.

Nicotine also displays signals in the mid- and low-field regions (2 – 4 ppm). These resonances were however not suitable for quantitation because of significant signal overlap. The relative concentrations of the major components (PG and VG) are also generally much higher than that of nicotine and, accordingly, have larger relative intensities in the NMR spectra. Publications where NMR was employed to investigate vape liquid composition made use of either 1,2,4,5-tetrachloro-3-nitrobenzene, DMSS or TSP as the internal standard, and DMF, MeOD or D_2O as the deuterated solvent. The DMF and 1,2,4,5-tetrachloro-3-nitrobenzene combination was reportedly better suited for general analysis since proton exchange between the internal standard, the excipient and DMF was negligible.[22-24] In this study, TSP was chosen since the focus was on water soluble nicotine, PG and VG concentrations. NMR based studies using TSP as an internal standard afforded concentrations which were in close agreement to those determined by LC-MS for the same samples. In addition, the samples for NMR analyses included a potassium phosphate buffer at pH 7.4, which ensured that nicotine in solution was predominantly in its neutral form. For nicotine, PG and VG quantitation, representative signals were chosen and integrated against the internal standard, TSP.

The procedure for preparing and analysing the vape samples was adapted from previously reported studies, involving the quantitation of nicotine in tobacco liqueur and vape liquid by ^{1}H NMR spectroscopy. In the referenced articles, 1 mM TSP was used as the internal standard.[22] For this study 10 mM TSP was used instead, since it was found that lower TSP concentrations lead to lower repeatability and linearity in the standard curves generated for the most abundant analytes. Due to the suppression of exchangeable protons and the drop-off in reliability of peak integration, the zgpr pulse sequence is typically not used for qNMR applications.[25] However, in this study we opted to use the zgpr pulse sequence since the the primary signals of interest were far removed from the H_2O resonance at which the presaturation frequency is applied. The signals of interest were also non-exchangeable protons dissolved in a largely D_2O-based buffer, which benefits from some form of water suppression. Additionally, there were a number of reports indicating the compatibility of zgpr pulse program with qNMR quantitation.[26-28]

The relaxation delay (D1) was initially set to 10 sec and incrementally reduced to 1 sec while monitoring the change in the relative integration of IS to analyte signals of interest. Since the signals of interest seemed to be relatively unchanged by a changing D1, the shorter D1 of 1 sec was used to reduce experiment time and allow for better sample throughput. The resultant calibration curves showed linear trends with good correlation ($R^2 > 0.99$) across the prepared concentrations using the shorter D1 value, with the signals of interest integrated against TSP. A summary of the calibration curves of the major components of the vape liquids are shown in Table 1.

Standard solutions were prepared in triplicate and the average peak integrals relative to TSP were used to construct the calibration curves using Topspin 3.2. For each of the standard solutions analysed in triplicate, there was variability in the standard deviation across the three technical repeats, ranging from 2-120%. This may have been due to the vape liquids being dispensed and measured by volume and, since vape liquids are relatively viscous solutions, this may have affected the standard deviation and subsequent repeatability. Future analysis should therefore include warming the vape samples in water bath at a standard temperature for a set period before weighing out the samples for analyses. Another important factor was the concentration range over which the calibration curve was constructed as well as the

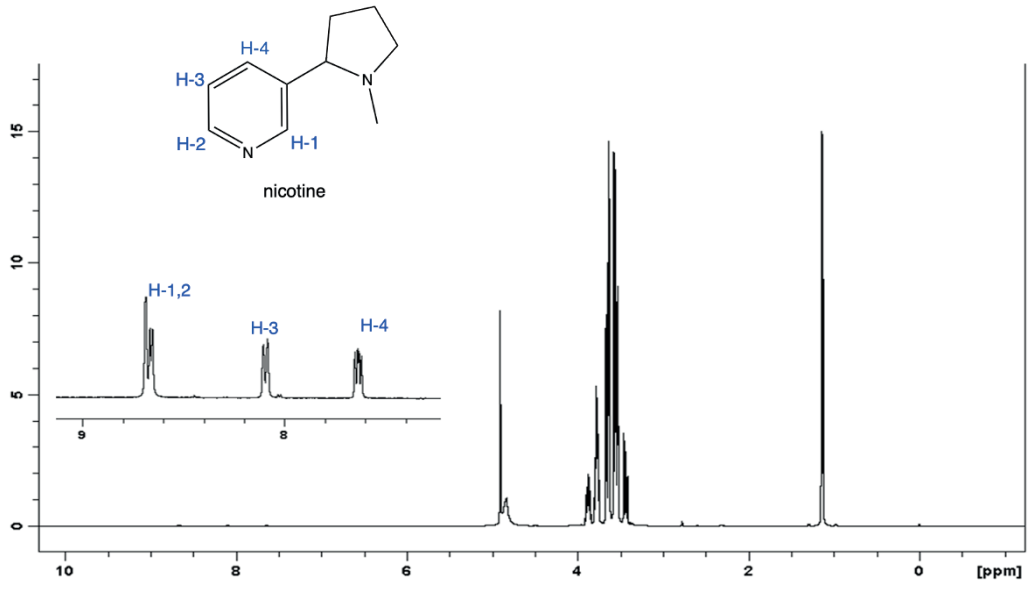


Figure 1. ^{1}H NMR spectrum of a 60 µL vape liquid sample dissolved in 60 µL D_2O and 480 µL distilled water and 60 µL NMR, with an expansion of the 7 – 9 ppm region of the spectrum.

Table 1. Calibration curve data generated by qNMR analysis for PG, VG, and Nicotine standard solutions.

Analyte	Region integrated (ppm)	Concentration Range (mg/mL)	LOQ (mg/mL)	LOD (mg/mL)	Linearity
PG	3.82-3.92	0.1-1 000	1.0	0.1	0.999
VG	3.73-3.81	0.1-1 000	1.0	0.1	0.9905
Nicotine	8.65-8.72	0.05-10	1.0	0.05	0.9971

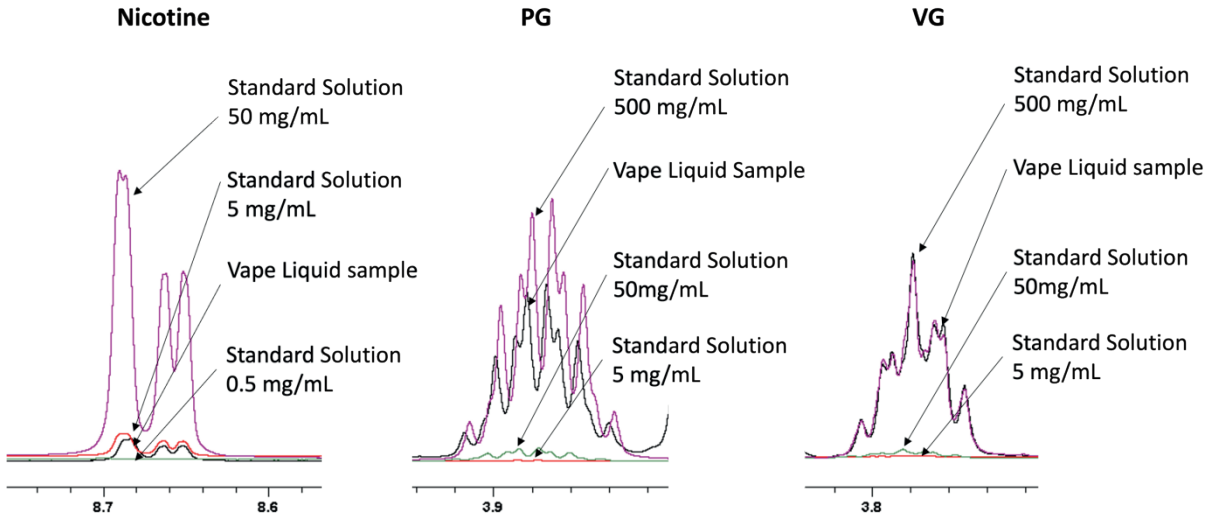


Figure 2. ^{1}H NMR spectra of standard solutions of Nicotine, PG and VG.

purity of the standards. To this end, we determined the HLPC purity of the nicotine standard solutions which was found to be >85% for all solutions tested, over the range tested.

The concentration range chosen for the standard curves reflect the range over which the analytes are typically found in vape liquid products. The limit of detection (LOD) and limit of quantitation (LOQ) for each analyte was determined by measuring the signal-to-noise ratio at the lower limit of the concentration ranges. The LOD and LOQ are used to define the lowest concentration of an analyte that can be reliably detected or quantified, respectively. It is determined by the signal-to-noise ratio (S/N), where a region in the ^{1}H NMR spectrum defined in Table 1, is measured against a region where there are no ^{1}H signals present. The LOD generally indicates the point at which the analyte signal becomes distinguishable from the background noise, at a signal-to-noise ratio of 3:1 or higher. On the other hand, the LOQ is the lowest concentration or amount of an analyte that can be reliably measured and quantified. It is was determined at a higher signal-to-noise ratio of 10:1 using the ^{1}H spectra of the calibration standards. The LOD and LOQ for nicotine from qNMR analysis was found to be 1.0 mg/mL and 0.05 mg/mL respectively (Table 1). Notably, previous studies utilising HPLC to quantify nicotine have reported a LOD and LOQ of around 0.07 µg/mL and 0.3 µg/mL, respectively.[29,30] These values are substantially lower (approximately 14 000 times lower for the LOD) than those obtained using qNMR. However, in this study, we demonstrate that NMR is better suited for the concentration ranges of interest.

Figure 2 shows the ^{1}H NMR spectra of the selected resonances of nicotine, PG and VG in vape liquid and in standard solutions (0.5, 5, 50 and 500 mg/ml), referenced against 10 mM TSP, where the intensities of TSP in each set of overlayed spectra are matched. Each of the signals representative of Nicotine, PG and VG had no significant overlap with signals from other components. In Figure 2, the NMR spectrum of a commercial nicotine containing vape liquid sample is displayed, together with the spectra obtained from standards. The vape liquid sample had the concentration of nicotine clearly reported on the label as 8 mg/mL, and further indicated a 1:1 ratio of PG to VG as well as the presence of unspecified flavourings. From the ^{1}H NMR analysis, it was found that the nicotine concentration was 1.2 mg/mL and the ratio of PG to VG was 1:1.6 (with concentrations of 298.1 mg/mL and 472.4 mg/mL for PG and VG, respectively).

This degree of incorrect labelling was generally observed for the various samples tested. Some samples that had indicated a 1:1 ratio of PG to VG were found to contain twice as much VG relative to PG and, only one of 20 samples tested had a PG:VG ratio that corresponded to the label. All of the nicotine containing samples that were analysed contained less nicotine than indicated on the label, ranging between 2-33% of the indicated amount. Some brands of vape liquid indicated only a number for Nicotine strength with no units. In these cases, it was assumed that the suggested strength was measured in mg/mL. Ethylene glycol, which was of concern because of its known toxicity, was not detected in any of the samples tested. Some samples presented aldehyde signals in the region of 9.80-9.85, these were assumed to be due to flavouring compounds such as vanillin and ethyl vanillin. Samples labelled as nicotine free generally did not contain nicotine (86%) or nicotine concentrations where below the LOD. Interestingly, previous studies, all conducted outside of South Africa, have found nicotine in many of the nicotine free products that were analysed.[7,9] Most of these reports used methods of analysis with improved LODs relative to 1D ^{1}H NMR. However, the reported values were greater than 1 mg/mL, which is greater than the LOD determined for nicotine in this study. A comparison of the label and NMR quantities are shown in Table 2.

If information regarding propylene glycol (PG), vegetable glycerin (VG) or nicotine concentrations was not shown on the product label, then the sample data was recorded as "not indicated" (NI). Samples that indicated nicotine concentration as 0 (on the label) are considered nicotine free.

Based on the numerous reports where nicotine was commonly found in vape liquid samples which were branded as nicotine free, it is surprising that only 1 of the 7 South African samples contained detectable levels nicotine. A study which looked at identifying counterfeit, nicotine free vape samples found that 51.7% of Nigerian (n = 29), 3.7% of Chinese (n = 27), 1.6% of American (n = 61) and 0% (n = 8) of British nicotine free vape liquid samples contained nicotine.[7] The study also found a correlation between sample authenticity and the presence of nicotine, where non-nicotine containing sample

Table 2. Nicotine, PG, and VG content from product labels compared to Nicotine, PG, and VG content as quantified by ^{1}H NMR analyses.

Sample Number	From Product Label		From ^{1}H NMR analyses	
	PG/VG	Nicotine (mg/mL)	PG/VG	Nicotine (mg/mL)
1	NI	35	1.08	0.7
2	NI	35	1.02	0.6
3	NI	18	1.05	4.2
4	NI	0	1.07	0.0
5	NI	12	1.34	2.0
6	NI	0	1.35	0.0
7	1	8	0.48	1.2
8	1	0	0.54	0.0
9	1	NI	1.24	0.0
10	NI	0	0.69	0.0
11	0.43	NI	0.55	0.0
12	0.43	18	0.28	4.0
13	0.43	0	0.28	0.0
14	0.43	18	0.30	3.6
15	0.43	0	0.30	0.0
16	0.43/2.33	0	0.36	0.0
17	NI	3	0.38	1.0
18	1	18	0.73	6.0
19	1	18	0.70	5.8
20	1	0	1.24	4.3

Table 3. Nicotine quantitation of standard vape liquid samples.

Concentration of Nicotine (mg/mL)	Concentration of Nicotine using NMR-based calibration curve (mg/mL)	Standard deviation (n = 3)	Percentage difference (%)
35	36.54	± 4.25	4.4
18	22.75	± 2.85	26.4
8	4.70	± 0.16	41.3
0	ND	-	-

which were found to contain nicotine were generally also counterfeit, with 81.3% of counterfeit products containing Nicotine.[7] The absence of nicotine in the South African samples could therefore support that the vape liquid samples were authentic and locally produced. It may also suggest that South African manufacturers generally adhere to nicotine policy and do not include nicotine in samples which are labelled as nicotine free. The ^{1}H NMR supported absence of nicotine in the nicotine free samples that were tested was further probed through the evaluation of nicotine concentrations in standard vape liquid samples. To this end, vape liquids were prepared at 35, 18, 8 and 0 mg/ml nicotine concentrations in only VG. The purity of the nicotine reagent used to prepare the standard samples was confirmed using HPLC. The standard samples were then subjected to NMR analysis in triplicate using the same method applied to the commercial products. The results of NMR nicotine quantitation for the standard samples are shown in Table 3.

Vape liquids with varying nicotine content were prepared in-house, using vegetable glycerin (VG) as the excipient only. The nicotine free sample contained VG only. qNMR Spectra were recorded in triplicate, with the difference between the known concentration and that determined by qNMR shown as the percentage difference.

For the standard vape samples, the nicotine concentrations determined by ^{1}H NMR spectroscopy were in good agreement with the prepared concentrations, where the 35 mg/mL samples were found to have an average nicotine concentration of 36.54 mg/mL, while the samples prepared at 18 mg/mL were found to contain an average of 22.75 mg/mL. A larger percentage difference was observed at the lower concentration of 8 mg/mL (41.3%), where samples prepared at 8 mg/mL were found to contain an average of 4.70 mg/mL nicotine. This result suggests that the lower nicotine concentrations determined for some of the commercial vape liquid samples may have been underestimated, and that the actual nicotine concentration may have been 40% greater. However, after applying such an adjustment, the tested local vape liquid samples would still generally have lower nicotine concentrations compared to the nicotine concentration indicated on the labels.

Published studies have reported nicotine concentrations varying from the concentrations indicated on the product label. Using ^{1}H NMR and GC-MS analysis, it was found that in 54 samples the average nicotine concentration was 11 mg/mL, and that most of the products had less than half the indicated nicotine concentration. In the "nicotine free" vape liquids, some samples were found to contain significant amounts of nicotine. The NMR based report indicated that 18 of 23 "nicotine free" samples were found not to contain nicotine.[19] Another study using GC-MS to quantify nicotine and other vape liquid components reported that nicotine was detected in 6 of 10 "nicotine free" vape liquids, with concentrations comparable to commercially available low dose vape liquids.[9] Other publications have shared similar findings, where significant levels of nicotine are detected in "nicotine free" vape liquid samples.[7,13]

CONCLUSION

Using an NMR-based approach, local vape liquid samples were analysed for nicotine, PG, VG concentrations. It was found that the vape liquid samples contained between 2-33% of the indicated nicotine concentration and that the PG:VG ratio was significantly different to that indicated on the label. Additionally, only one of the vape liquid samples which was labelled as nicotine free, was found to contain nicotine (4.3 mg/mL) at a level above the ^{1}H NMR LOQ. The fact that the analysed samples generally contain less nicotine than indicated on product labels (2-33%) and that most of the nicotine free products are indeed free of nicotine (87%), are valuable insights into a local market for which there is currently little regulation and no reported analytical data. It should however be noted, that while these findings provide an initial indication that there are label claim discrepancies, the relatively small and exploratory sample set limits broader generalization. These results therefore highlight a potential public health concern and underscore the need for larger, statistically representative studies to more comprehensively evaluate vape liquid composition and labelling accuracy in for products on the South African market.

SUPPLEMENTARY MATERIAL

Supplementary material is available online as a separate document.

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AUTHOR CONTRIBUTIONS

Maryam Fredericks: HPLC data collection, Analysis, Writing-Review. **Marwaan Rylands**: Conceptualisation, qNMR data collection, Writing-Original Draft, Editing and Project Administration.

DECLARATION OF COMPETING OR FINANCIAL INTERESTS

The authors declare that they have no known competing financial interest or personal relationship that could have influenced the resulted of this study.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES

The authors declare that AI was not used in the preparation of this manuscript or in the analysis of the data.

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