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Technical Note

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The Analysis of Illicit Methaqualone Containing Preparations by Gas Chromatography–Mass Spectrometry for Forensic Purposes

ABSTRACT: A validated gas chromatographic–mass spectrometric method for quantitative analysis of methaqualone (MTQ) in illicit preparations is reported. The method proved to have a coefficient of variation of below 5%. Four batches of seized tablets, two pairs with similar imprints, were analyzed. It was found that the average MTQ concentration in all four batches of tablets differed significantly ($p = 0.01$) rendering it impossible to conclude that, on the basis of MTQ concentration alone, the batches with a similar logo originated from the same manufacturer or manufacturing batch. Conversely, it can be said that in this case, the four batches originated from either different clandestine laboratories or manufacturing batches.

KEYWORDS: forensic science, forensic chemistry, mandrax, methaqualone, quantitative analysis, gas chromatography–mass spectrometry

A validated gas chromatographic–mass spectrometric (GC–MS) method for quantitative analysis of methaqualone (MTQ) is reported. The method was used to comment on the origin of four batches of MTQ tablets, two pairs with similar logos, seized in different areas of South Africa.

Illicit manufacturing of MTQ preparations is of great concern internationally. MTQ belongs to a group of compounds called the quinazolidines and is listed in the South African Drug and Drug Trafficking Act (1) and the Medicines and Related Substances Control Act (2). The number of seizures that involved MTQ tablets showed a dramatic increase from 2400 in 1999 to 8351 in 2003. Seizures in 2003 involved just over 1.3 million tablets, and an approximate additional 5000 kg of tablet pieces and powder. The South African street name for MTQ containing tablets is "mandrax." Figure 1 summarizes this trend in South Africa over a period of 5 years. All seized MTQ tablets are classified according to their logo, color and dimensions, and the detailed information is then entered into the South African National Forensic Drug Intelligence Database (NFDID), compiled since 1999. The information is published yearly as a Logo Index (3).

Forensic chemists are frequently confronted with the question of whether preparations seized, during different raids or from drug sellers, originate from the same manufacturer. The answer to this question lies in the combination of micro-, macroscopic analysis (4,5), and chemical "fingerprinting" (5,6). The latter refers to qualitative and quantitative chemical analysis of the major and trace constituents.

A number of chromatographic techniques have been used for qualitative analysis of MTQ containing preparations. The use of response index (7) added a second dimension to the identification of drugs, next to retention time. The response index of an eluting peak is the ratio of its response toward nitrogen–phosphorous— and flame ionization detection. Utilizing response index in combination with retention time increased the analytical selectivity toward chemical classes. Baker et al. (8) as well as Reuland and Trinler (9) concluded that the use of relative retention times alone, in HPLC analysis of drugs, resulted in an identification success rate of only 9%. A combination of retention time and absorbance ratios (absorbances at 254 and 280 nm) increased the identification success rate to 95%. Saferstein and Chao (10) recommended the use of a combination of electro and chemical ionization sources to control the complexity of the mass spectra produced.

Identification of precursors and by-products in MTQ and mecloqualone containing mixtures was found to be essential by Angelos and Meyers (11), in the identification of synthetic routes used during illicit manufacturing of quinazolinones. A combination of gas and liquid chromatographic, mass spectrometric, infrared and nuclear magnetic resonance techniques were used in this study.

Besides the major component, MTQ-containing tablets seized in South Africa frequently contains active compounds such as diphenhydramine (DPH) and diazepam. The concentration of these minor constituents, in combination with major component concentrations, can therefore potentially be used for purposes of "fingerprinting," as far as manufacturer or manufacturing batch is concerned. This work presents a validated GC–MS method for quantitation of MTQ in illicit mixtures. This method was subsequently applied to four batches of seized tablets, two pairs with similar logos, for the purpose of assessing the origin of manufacture.

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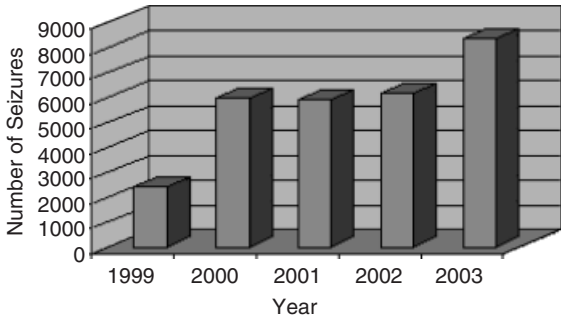

FIG. 1—*The number of methaqualone seizures analyzed by the South African Police Service Forensic Science Laboratory over a period of 5 years.*

Methodology

Reagents and Chemicals

Chloroform (HPLC grade) and anhydrous sodium sulfate (analytical grade) were obtained from Sigma–Aldrich (Fluka, Buchs, Switzerland). The starch and lactose were purchased from Separations (ChemService, West Chester, PA). Certified analytical standards of MTQ and DPH were used (BP, London, UK).

Equipment

A HP6890 GC system fitted with a HP7683 auto injector and a HP5973 mass-selective detector (MSD) (Agilent Technologies, Palo Alto, CA) was used for mass spectrometric analysis. Data collection and integration was performed with HP Chem Station software. A DB-5MS (J&W Scientific, Agilent Technologies, Palo Alto, CA), capillary column was used (30 m × 250 μm; d_f 0.25 μm). The MSD tune was performed with perfluorotributylamine for masses 69, 219, and 502 using the auto tune option.

Sample Preparation and Extraction Procedure

For MTQ and DPH quantitation, seized tablets as well as the tablets pressed for recovery studies were pulverized and homogenized with a mortar and pestle. One gram was transferred into a test tube (10 mL). Chloroform (5 mL) was added and the mixture vortexed for 10 sec. The supernatant was filtered through anhydrous sodium sulfate. An aliquot of the supernatant (1 μL) was then transferred to a vial (2 mL). After the sample was dried under a stream of nitrogen, it was reconstituted with chloroform (1 mL), and subjected to GC–MS analysis.

GC–MS Detection Procedure

A 1 μl of the chloroform extract was injected in splitless mode. The splitter was opened to vent at 1.5 min. The inlet temperature was set at 250°C and helium carrier at a constant flow-rate of 1 mL/min. The initial oven temperature was set at 100°C and was ramped immediately after injection to 280°C at a rate of 10°C/min, with a final isotherm of 5 min. The total chromatographic time was 23 min. The transfer line temperature was set at 280°C and that of the quadrupole at 106°C. The source temperature was 230°C. A solvent delay time of 4 min was used to allow for solvent elution before the source was turned on. All mass spectra were recorded at 70 eV. Chromatograms were recorded in the scan mode (40–450 *m/z*) to identify the analytes in standard solutions and ascertain their retention times. Quantitation was performed with extracted ions, recorded in full scan mode. The mass-to-charge ratios (*m/z*) of the ions that were used for quantitation were 91, 235, and 250 *m/z* for MTQ and 58, 73, and 165 *m/z* for DPH.

FIG. 2—*Photograph of a typical tablet, with an imprint on one side only, from cases 1 and 4 seized in the Southern and Western Cape provinces in South Africa, respectively.*

Standards and Calibration Curves

Stock solutions of MTQ (100 mg/L) and DPH (100 mg/L) were made in chloroform. Calibration standards with concentrations ranging from 10 to 100 mg/L were made up by dilution with chloroform Concentrations of the calibration standards ranged from 10 to 100 mg/L with 10 mg/L intervals. External standards used for the determination of MTQ and DPH in the four test cases ranged from 10 to 60 mg/L with 10 mg/L intervals.

Tablets were manufactured in-house with a starch–lactose powder mixture (36% lactose, 8.5% starch, 5% talc, and 0.5% magnesium stearate) (12) to perform recovery studies. The starch–lactose mixture (1 g) was spiked with 300 μL of the MTQ stock solution (100 mg/L) to obtain a final concentration of 0.03 mg MTQ per gram tablet. The mixture was homogenized, and tablets (1 g) were pressed from the homogenized mixture. The tablets were then subjected to the same extraction procedure as the seized tablets.

Sampling Procedure

Four groups, 30 tablets each (T1–T4), were randomly selected from four batches of seized "mandrax" tablets destined for destruction. Sample groups T1 and T4 came from batches seized in the Southern and Western Cape provinces of South Africa, respectively. They had similar masses and appearances with the same imprint on only one side—and were classified under the same NFDID code. A photograph of a typical tablet is shown in Fig. 2. Sample groups T2 and T3 came from batches seized in the Northern Cape and Gauteng provinces of South Africa, respectively. Again they were classified under the same NFDID code and had similar masses and physical appearances as well as the same imprints on both sides of the tablets. A photograph of the two sides of a typical tablet is shown in Fig. 3.

Assay Validation

During the validation process parameters such as limit of detection (LOD, 3σ above baseline), limit of quantification (LOQ, 10σ above baseline), linearity, relative standard deviation, recovery, and reproducibility were addressed. Four independent analysts recorded the data in duplicate, i.e., the data presented are those of the average duplicate analyses of four analysts. In-house manufactured "tablet standards" were used for validation of the extraction procedure.

FIG. 3—*Photograph of a typical tablet, with an imprint on both sides, from cases 2 and 3 seized in the Northern Cape and Gauteng provinces in South Africa, respectively.*

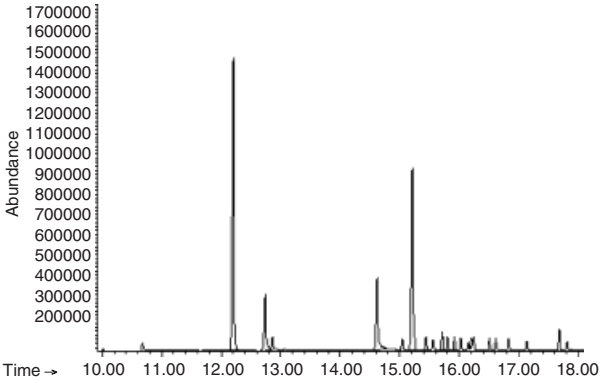

FIG. 4—*A total ion chromatogram, obtained from a cocktail of illicit compounds.*

Statistics

Standard parametric statistical analysis using ANOVA and the Studentized range were used to evaluate the differences between batches.

Results and Discussion

Method Validation

A total ion chromatogram, obtained by analyzing a cocktail of illicit compounds, containing all the isomers of MTQ (isomers I–XII) (13), as well as DPH, is shown in Fig. 4. All MTQ isomers and DPH were sufficiently resolved for this chromatographic method to be used in quantitative analyses of MTQ (isomer I) and DPH. MTQ and DPH eluted at 12 h 20 min and 15 h 21 min, respectively. The electron ionization mass spectrum of MTQ (70 eV) is shown in Fig. 5.

A calibration curve of the extracted ion response was used to set-up the calibration graph for MTQ. The calibration curve was found

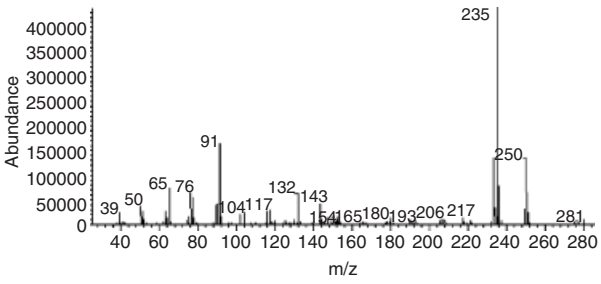

FIG. 5—*An electron impact mass spectrum of methaqualone (70 eV).*

TABLE 1—*Characteristics of the mass and concentration (mass percentage, mg MTQ/mg tablet) data for methaqualone and diphenhydramine in the 30 tablets analyzed in each of the sample groups.*

	Test Case 1 (T1)			Test Case 2 (T2)			Test Case 3 (T3)			Test Case 4 (T4)		
	Tablet Mass (mg)	MTQ	DPH	Tablet Mass (mg)	MTQ	DPH	Tablet Mass (mg)	MTQ	DPH	Tablet Mass (mg)	MTQ	DPH
Mean	1051.19	0.39	0.050	496.47	6.11	0.044	497.15	10.68	0.106	1001.40	15.24	x
Median	1052.42	0.41	0.047	496.32	6.14	0.044	495.70	10.86	0.104	1012.10	15.35	x
Interquartile range	1050.12	0.41	0.047	496.52	6.21	0.043	495.92	10.96	0.104	1007.75	15.46	x
SD	31.56	0.23	0.023	10.23	0.87	0.013	13.43	2.22	0.039	35.62	2.95	x
CV	3.00%	59%	46%	2.06%	14%	31%	2.70%	21%	36%	3.56%	19%	x
Skewness	0.08	− 0.15	1.30	0.26	− 0.51	0.23	0.97	− 1.04	1.77	− 0.68	− 0.47	x
99% CI	1036.5–1066.04	0.28–0.50	0.039–0.060	491.66–501.28	5.70–6.25	0.037–0.050	490.83–503.47	9.64–11.73	0.088–0.125	984.65–1018.15	13.85–16.62	x

MTQ, methaqualone; DPH, diphenhydramine; CV, coefficient of variation.

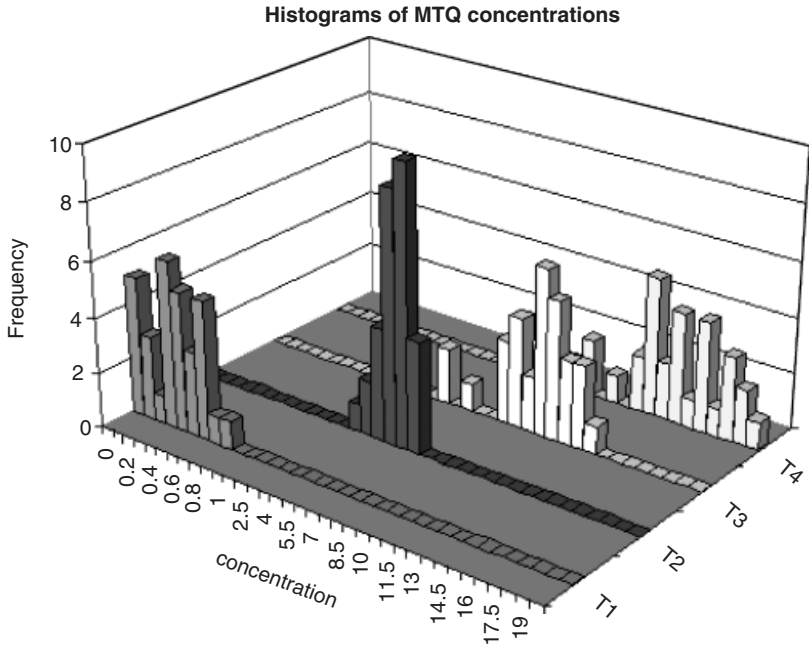

FIG. 6—*Histograms of the concentration distribution for methaqualone in all four sample groups.*

to be linear over a concentration range of 10–100 mg/L (r^2 = 0.9947) and the LOD and LOQ to be 0.0018 and 0.0687 mg/L, respectively. The LOQ was well below the anticipated working range of 20–100 mg/L. The recovery at the 0.03 mg/g tablet level was found to be 92%, and the coefficient of variation (CV) of eight in-house manufactured tablets was 4.8% at a concentration of 0.03 mg/g tablet level. The linear correlation coefficient was 0.9947.

Case Study

MTQ was detected and quantified in all four sample groups while only test cases T1, T2, and T3 contained DPH. Table 1 summarizes the characteristics of the concentration (mg MTQ/mg tablet × 100) data for MTQ and DPH in the 30 tablets analyzed in each of the four test cases. The histograms of the concentration distribution for both MTQ and DPH are shown in Figs. 6 and 7, respectively.

The large within-batch coefficients of variation demonstrated in the MTQ concentration of the sample groups can typically result from poor homogenization procedures in clandestine laboratories. This phenomenon poses a toxicity risk, as drug abusers might unknowingly overdose. A large degree in variation in the CV between batches was also recorded in literature by Sher (14) for nine different MTQ-containing formulations. The variation between

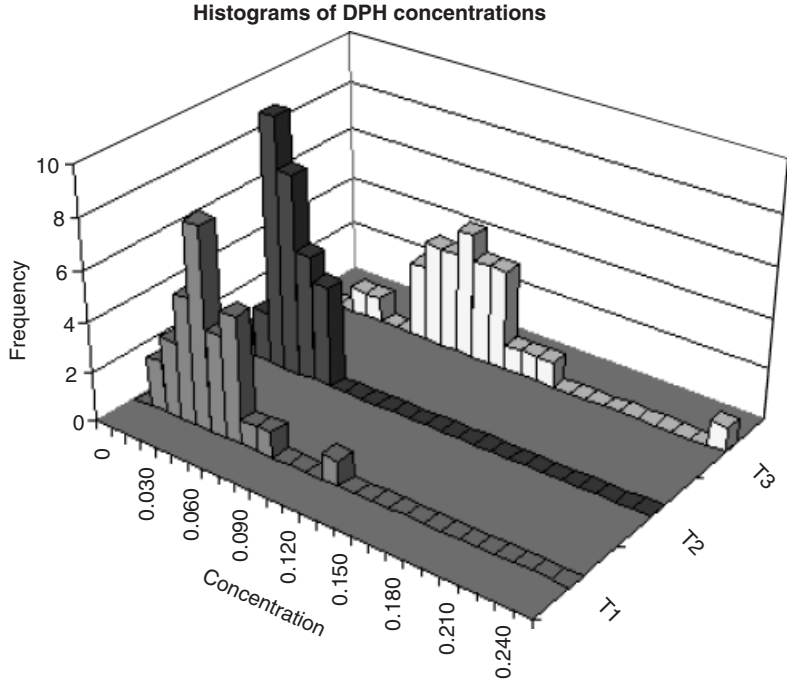

FIG. 7—*Histograms of the concentration distribution for diphenhydramine in test cases T1, T2, and T3.*

batches should, however, be relatively small if they were from the same origin. In combination with the lack of overlap in the 99% confidence intervals, this indicated that the batches were different.

The concentrations of DPH in the sample groups also demonstrated a large CV. The 99% confidence interval shows a large degree of overlap between sample groups T1 and T2. We are, however, of the opinion that the data provided by the MTQ measurements are superior to that provided by the DPH measurements. MTQ is the main illegal constituent of the tablets and the characteristics of the DPH data ranges do not reflect true Gaussian distribution as well as the MTQ data ranges. This can be depicted in the difference in the mean and medians of the data and its skewness. The DPH measurements do, however, have qualitative value, in that it clearly identifies sample group T4 to be different from all the others.

A one-way ANOVA analysis was performed on the MTQ concentrations obtained from all four sample groups. The same analysis was repeated for the DPH concentrations of groups T1 to T3. The analysis was set out to demonstrate differences in all four groups simultaneously and not just between the groups sharing the same physical characteristics. We propose that the concentrations of DPH and MTQ varied independently from each other and a simultaneous factorial ANOVA analysis was, therefore, not sensible. In both cases the ratio of the variation between groups to the variation within groups (F (MTQ) = 335 and F (DPH) = 49) were much larger than the critical ratio ((F_{crit} (MTQ) = 3.96 and F_{crit} (DPH) = 4.86) at $p = 0.01$. It can, therefore, be concluded that none of the batches share a common origin.

A studentized range analysis was subsequently performed on the individual groups. The critical difference between groups was calculated to be 1.29 for MTQ. It could, therefore, be concluded that all four groups were significantly different from each other as far as the MTQ concentration is concerned. The critical difference in the DPH groups was calculated to be 0.018 and, therefore, statistical significance could not be demonstrated between the T1 and T2 groups in terms of DPH concentration.

Batches T1 and T4 had the same weight and logos, as did batches T2 and T3. The between batch variation from the same manufacturer is unknown, however, it is unlikely to be as high as the differences reflected by the means of the MTQ concentration in Table 1. Statistical analysis of MTQ concentration data ($p = 0.01$) could not support the superficial conclusion, based on weight and appearance, that either T1 and T4, or T2 and T3, have the same origin. Conversely it can be said that in this case, the four batches originated from either different clandestine laboratories or manufacturing batches. It is also known that different manufacturers use the same logo that may be fashionable at the time. The fact that test case T4 did not contain any DPH suggests that the two cases T1 and T4, even though they had identical logos, were from different laboratories. The analysis of MTQ isomers did not yield additional information regarding the origin, since all tablets contained only MTQ isomer I. The presence of precursors or reaction by-products might augment the "fingerprinting" process.

Acknowledgments

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