UNODC
United Nations Office on Drugs and Crime

Recommended methods for **the identification and analysis of methaqualone/mecloqualone**

FOR USE BY NATIONAL DRUG ANALYSIS LABORATORIES

Laboratory and Scientific Section
UNITED NATIONS OFFICE ON DRUGS AND CRIME
Vienna

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UNTIED NATIONS
New York, 2010

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Contents

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*Contact details of named individuals can be requested from the UNODC Laboratory and Scientific Section (P.O. Box 500, 1400 Vienna, Austria).

1. Introduction

1.1 Background

Methaqualone, a quinazolone derivative was first synthesized in 1951, and used medically as a hypnotic for the short-term treatment of insomnia, and also as a recreational drug. It acts as a central nervous system depressant in a similar manner to barbiturates. It was commonly marketed as Quaaludes and Mandrax (containing methaqualone base 250 mg, and diphenhydramine hydrochloride 25 mg) in the 1960s and 1970s and also as Malsed, Malsedin, and Renoval in the United Kingdom (see the *Multilingual Dictionary of Narcotic Drugs and Psychotropic Substances under International Control* (MLD), www.unodc.org). Due to abuse, addiction and a higher potential to create dependence than the barbiturates, methaqualone is no longer prescribed in medicine and it is under international control (Schedule II of the Convention on Psychotropic Substances 1971). The detrimental effects of hypnotic dependence on methaqualone arise particularly from the persistence of effects such as ataxia, dysarthia, mental impairment, confusion and poor judgement. Side effects of treatment with methaqualone or its hydrochloride in therapeutic doses include headaches, hangovers, dizziness, drowsiness, anorexia, nausea and gastro-intestinal discomfort, dry mouth, restlessness and sweating. Skin reactions have also been reported. Methaqualone is now only largely illicitly produced in clandestine laboratories. Its main use is as a recreational drug taken orally as a tablet formulation and often smoked when mixed with cannabis (marijuana).

Mecloqualone, an analogue of methaqualone, was synthesized in 1960 and was available as a legitimately dispensed hypnotic, especially in France under the proprietary names Nubarene and Casfen (refer to MLD, www.unodc.org) and some other European countries. It was never as widely used as methaqualone and is also no longer prescribed because of concerns about its potential abuse and overdose.

1.2 Purpose and use of the manual

The present manual is one in a series of similar UNODC publications dealing with the identification and analysis of various types of drugs under international control. These manuals are the outcome of a programme pursued by UNODC since the early 1980s, aimed at the harmonization and establishment of recommended methods of analysis for national drug analysis laboratories.

In line with the overall objective of this series of UNODC publications, the present manual suggests approaches that may assist drug analysts in the selection of methods appropriate to the sample under examination and provide data suitable for the purpose

at hand, leaving room also for adaptation to the level of sophistication of different laboratories and the various legal needs.

Any new method that is about to be used in the reader's laboratory must be validated and/or verified prior to routine use.

The methods described here should be understood as guidance, that is, minor modifications to suit local circumstances should normally not change the validity of the results. The choice of the methodology and approach to analysis as well as the decision as to whether or not additional methods are required remain with the analyst and may also be dependent on the availability of appropriate instrumentation and the level of legally acceptable proof in the jurisdiction within which the analyst works.

Attention is also drawn to the vital importance of the availability to drug analysts of reference materials and literature on drugs of abuse and analytical techniques. Moreover, the analyst must of necessity keep abreast of current trends in drug analysis, consistently following current analytical and forensic science literature.

UNODC Laboratory and Scientific Section welcomes observations on the contents and usefulness of the present manual. Comments and suggestions may be addressed to:

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All manuals, as well as guidelines and other scientific-technical publications may be requested by contacting the address above.

2. Illicit trafficking of methaqualone/mecloqualone

2.1 Illicit manufacture

The synthesis of methaqualone usually involves uncomplicated one and two step reactions that are easily performed in clandestine laboratories [1]. A one-step reaction is carried out by refluxing anthranilic acid, acetic acid (or acetic anhydride) and *o*-toluidine (o-chloroaniline for mecloqualone). Polyphosphoric acid is added to remove water. Purification is accomplished by dissolving the solid residue in methanol and precipitation of the hydrochloride salt from a methanol-diethyl ether solution.

The two-step reaction involves the preparation of *N*-acetylanthranilic acid from anthranilic acid and acetic anhydride followed by condensation with *o*-toluidine in the presence of phosphorus trichloride. Substituting *o*-chloroaniline for *o*-toluidine would produce mecloqualone.

According to the International Narcotics Control Board (INCB), the cumulative global seizures of the precursors since 2000 were 10.4 metric tons of *N*-acetylanthranilic acid and 34.5 metric tons of anthranilic acid, enough to produce approximately 42.8 metric tons of methaqualone. The total seizures of these precursors have varied dramatically from between 5 kg to 25.6 metric tons annually, since 2000. The largest multi-ton seizures have been reported in China (*N*-acetylanthranilic acid), Mozambique and South Africa (anthranilic acid).

Clandestinely produced methaqualone appears on the illicit market as a brown, grey or black tacky powder with 30-70 per cent purity. The colour depends on the amount of impurities present. Methaqualone is also used as a cutting agent for heroin and can be present in seizures of heroin up to a concentration of about 30 per cent.

2.2 Main trends and patterns in illegal trafficking

Data related to the use and seizures of methaqualone and mecloqualone, their chemical precursors and clandestine manufacture are sporadic and inconsistent making the analysis of trends difficult.

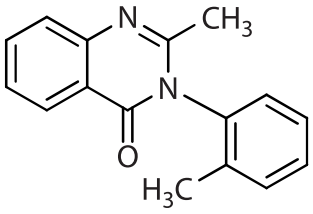
Currently there are no estimates of global lifetime or past-year use of this drug. However, most use is assumed to occur in South Africa. In 2006, a survey of students in Cape Town, South Africa aged 13-17 years found a lifetime prevalence of 2.6 per cent and a past-year use of 1.1 per cent for this drug. Treatment for the drug accounted for approximately 4 per cent of all drug treatment cases in South Africa in 2007.

Since 2000, only 22 countries have reported seizures of methaqualone, of which half were located in Africa's southern and central subregions. The cumulative amount seized globally since 2000 is approximately 37.5 metric tons, the majority of which was seized in 2001-2002. India (a large producer of methaqualone) and South Africa (a large consumer of methaqualone) account for 47 per cent and 44 per cent of global seizures respectively.

Since 2000, reports of illicit manufacture have come from only four countries: China, India, Kenya and South Africa. During that time, 58 laboratories have been reported dismantled, of which 93 per cent (54) were reported in South Africa. The number of clandestine methaqualone laboratories in South Africa has declined since their peak in 2003-2004 when 15 laboratories were dismantled on average per year.

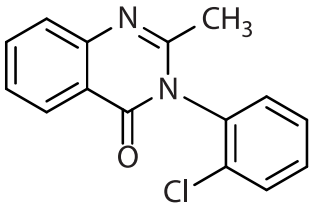
3. Description of the pure compounds

3.1 Methaqualone

2-methyl-3-*o*-tolyl-4-(3H)-quinazolinone Sch. II, 1971 	Empirical formula: $C_{15}H_{14}N_2O$ CAS No.: 72-44-6 Molecular weight: 250.7 g/mol Melting point freebase: 113-115˚C Melting point HCl salt: 235-237˚C Physical appearance: in its pure form, occurs as a white crystalline powder

Solubility in:	Water	Ethanol	Diethyl ether	Chloroform
Methaqualone base	insoluble	soluble	soluble	soluble
Methaqualone hydrochloride salt	soluble	soluble	insoluble	soluble

3.2 Mecloqualone

3-(*o*-chlorophenyl)-2-methyl-4-(3H)-quinazolinone Sch. II, 1971 	Empirical formula: $C_{15}H_{11}ClN_2O$ CAS No.: 340-57-8 Molecular weight: 270.7 g/mol Melting point freebase: 126-128˚C Melting point HCl salt: 239-241˚C Physical appearance: in its pure form, occurs as a white crystalline powder

Solubility in:	Water	Ethanol	Diethyl ether	Chloroform
Mecloqualone base	insoluble	soluble	soluble	very soluble
Mecloqualone hydrochloride salt	soluble	soluble	insoluble	soluble

Both methaqualone and mecloqualone may be encountered as powders, tablets or capsules and may be present as the base or the hydrochloride salt in these formulations.

4. Qualitative and quantitative analysis of materials containing methaqualone/mecloqualone

Generally, in attempting to establish the identity of a controlled drug in suspect material, the analytical approach must entail the determination of at least two uncorrelated parameters. One of which should provide information on the chemical structure of the analyte (for example, IR, MS; or tandem methods such as GC-MS).

It is recognized that the selection of these parameters in any particular case would take into account the drug involved and the laboratory resources available to the analyst. It is also accepted that unique requirements in different jurisdictions may dictate the actual practices followed by a particular laboratory.

4.1 Sampling

The principal reason for a sampling procedure is to permit an accurate and meaningful chemical analysis. Because most methods–qualitative and quantitative–used in forensic drug analysis laboratories require very small aliquots of material, it is vital that these small aliquots be representative of the bulk from which they have been drawn. Sampling should conform to the principles of analytical chemistry, as laid down, for example, in national pharmacopoeias or by regional or international organizations. For general aspects of qualitative sampling of multi-unit samples, refer to the *Guidelines on Representative Drug Sampling* (www.unodc.org). For seized material with obvious external characteristics, a sampling method based on the Bayes' model may be preferred over the hypergeometric approach.

The use of an approved sampling system also helps to preserve valuable resources and time by reducing the number of determinations needed. It is recognized that there may be situations where, for legal reasons, the normal rules of sampling and homogenization cannot be followed.

4.1.1 Extraction and sample preparation

Since methaqualone/mecloqualone exhibits are mostly from illicit manufacture, a simple extraction procedure may not work in all cases because of interference from raw materials, intermediates and by-products from clandestine routes. Nevertheless the following procedure provides a fast and simple way of isolating methaqualone or mecloqualone from most powder, tablet or capsule samples.

The representative sample of powder, tablet or capsule is suspended in 1M sodium bicarbonate solution. Methaqualone/mecloqualone (as the free base) may be extracted quantitatively with several portions of dichloromethane. The combined organic layers are filtered, dried over anhydrous sulphate and evaporated.

Due to the trace amounts of drug usually present on glassware and other equipment found in clandestine laboratories, the nature of the analysis should be geared towards conclusive analytical procedures rather than presumptive tests. Wash the syringe or glassware with a minimum amount of dichloromethane or methanol, concentrate to dryness under a stream of nitrogen and proceed with selected tests. Organic layers may be filtered, dried over anhydrous sulphate and reduced to dryness under a stream of nitrogen gas.

4.2 Examination

4.2.1 Colour test [2, 3]

(*a*) Cobalt thiocyanate test

Reagent A: 16 per cent hydrochloric acid solution
Reagent B: 2.5 g cobalt (II) thiocyanate in 100 ml of water

Method

Place a small amount of the suspected material into a test-tube. Add 1 drop of reagent A and 1 drop of reagent B. A blue colour indicates the possible presence of methaqualone or mecloqualone.

(*b*) Fischer-Morris test

Reagent A: Concentrated formic acid (88 per cent)
Reagent B: 5 per cent aqueous sodium nitrite solution

Method

Place a small amount of suspected material into a test tube. Add 7 drops of reagent A and then 5 drops of reagent B. Allow to stand for 1-2 minutes and then add 15-20 drops of chloroform. Shake, allow to stand and observe the colour of both layers.

Table 1. Results

Compound	Cobalt thiocyanate test	Fischer-Morris test	
		Water layer	Chloroform layer
Methaqualone	Blue	No colour	Yellow
Mecloqualone	Blue	No colour	Yellow
Cocaine	Blue	Faint green	Faint green
Phencyclidine	Blue	Tinged	Tinged
Caffeine	Blue	No colour	No colour
Heroin	Blue	Faint yellow	Yellow
Diphenhydramine	Blue	Yellow	No colour
Diazepam	Blue green	Faint yellow	Faint yellow

Analytical notes

- Positive results to colour tests are only presumptive indications of the possible presence of methaqualone or mecloqualone. Other controlled substances (cocaine, phencyclidine) and non-controlled drugs/precursors may produce a similar blue colour in the cobalt thiocyanate test.
- For the Fischer-Morris test, the order of addition and the ratio of drops are critical for obtaining the described results.
- A positive result is indicated by a yellow colour in 1-2 minutes. For methaqualone and mecloqualone, the yellow colour is extracted in the lower chloroform layer whilst the upper aqueous layer remains colourless. This is a true positive and should be differentiated from the yellow colour in the bottom layer and a yellow or other colour in the top layer with e.g. diphenhydramine, diazepam or ephedrine hydrochloride.
- A negative result is that in which the bottom layer does not have a yellow colour, regardless of the colour of the top layer.
- The sodium nitrite reagent works equally well at room temperature or even when cool. It has a long shelf life without special storage conditions/precautions.

4.2.2 Microcrystal test

Microcrystal tests [4] are quick, simple and extremely sensitive tests for the identification of substances. They involve the formation of crystals from the reaction of the target compound with a chemical reagent, followed by the analysis of the resulting crystals by means of a polarizing microscope and comparison with reference material, usually photographs of known crystals.

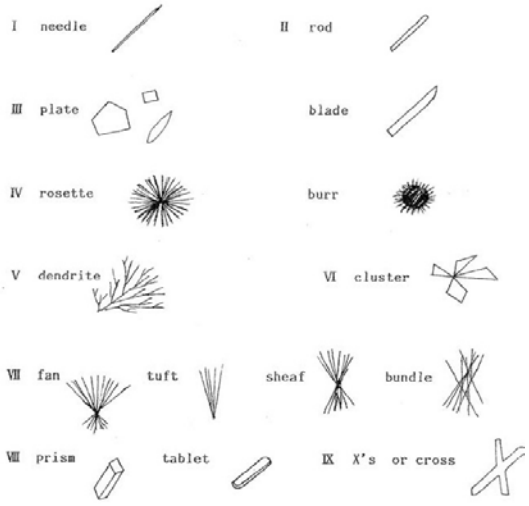
Method

The simplest form of the test consists of adding a drop of the reagent to the test substance, followed by observing and analyzing the crystals under a polarizing microscope. In order to maintain an accurate record, the characteristic features of the crystals should be described. The most accurate record of the test results is by photographs. In the absence of photography, a sketch of the crystal forms is helpful.

Hanging drop technique

This requires a cavity slide, a cover glass and the test reagent. A "microdrop", obtained from a very narrow glass tube (drawn from a glass tube), of the test solution is placed on the cover-slip and a "microdrop" of the reagent is added, the drop being stirred with slight scratching of the glass to promote the formation of crystals. A cavity slide is ringed with gum Arabica solution, inverted over the cover-slip, and re-inverted so as to leave a "hanging drop". Crystals are free to form slowly in a three-dimensional environment. Evaporation is prevented by the gum Arabic seal and this promotes regular, reproducible shapes.

Figure 1. Typical forms of microcrystals [4]



Typical forms of microcrystal can be classified into nine groups, using the descriptive terms above. In order to allow description of all types of microcrystals, adjectives such as irregular, fine or square-cut should be added to the basic terms.

Reagent I

Potassium permanganate solution: Transfer 25 g of potassium permanganate into a 100 ml flask; add 5 drops of phosphoric acid (88-90 per cent w/w) and make up to the mark with water.

Result

Methaqualone gives serrated plates (figure 1).

Reagent II

Sodium carbonate solution: Transfer 5 g of sodium carbonate in a 100 ml flask and make up to the mark with water.

Result

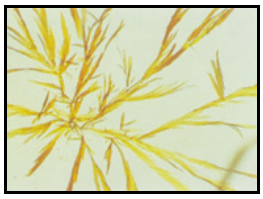
Methaqualone gives bunches of prisms (figure 1).

Reagent III

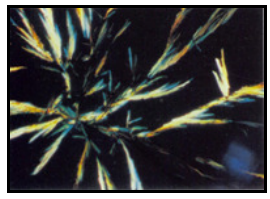
Iodine-iodine solution (I-KI B): Mix 0.5 ml of solution of 10 g of iodine and 35 g of potassium iodine in 100 ml water, 1.8 ml glacial acetic acid and 2.2 ml of syrupy phosphoric acid and 1.5 ml water.

Results

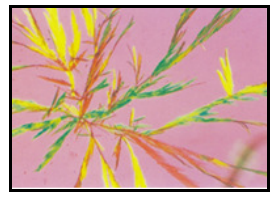
Figure 2. [reproduced from reference 4]



With polarized light



Polarized light with polarized crossed



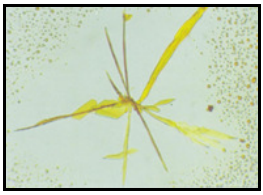
Polarized light with red plate

Reagent IV

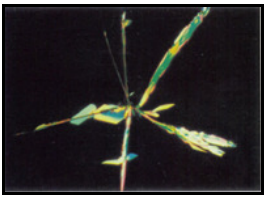
Picric acid solution: Prepare a solution of 0.03 g of picric acid in a mixture of 1ml of glacial acid and 5 ml of 40 per cent magnesium acetate solution.

Results

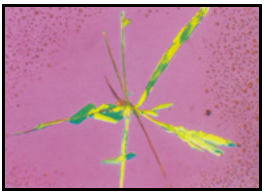
Figure 3. [reproduced from reference 4]



With polarized light



Polarized light with polarized crossed



Polarized light with red plate

4.2.3 Thin layer chromatography (TLC)

TLC is a commonly used technique for the separation and identification of illicitly manufactured drugs. It is inexpensive, rapid, sensitive (sub-milligram quantities of analyte required), flexible in the selection of both the stationary and mobile phase and amenable to a wide variety of substances, in base and salt form, ranging from the most polar to non-polar materials. A variety of visualization techniques can be used. However, in many countries it is not accepted as a single technique for drug identification.

TLC plates (stationary phases)

Coating: Silica gel G with a layer thickness of 0.25 mm and containing an inert indicator, which fluoresces under UV light wavelength 254 nm (Silica gel GF254).

Note: Plates prepared by the analyst must be activated before use by placing them into an oven at 120°C for at least 10 to 30 minutes. Plates are then stored in a grease-free desiccator over blue silica gel. Heat activation is not required for commercially available coated plates.

Typical plate sizes: 20 x 20 cm, 20 x 10 cm, 10 x 5 cm (the latter should be used with the 10 cm side vertical with the TLC tank).

Solvent systems (mobile phases, by volume)

System A: Cyclohexane : Toluene : Diethylamine (75 : 15 : 10 v/v)
System B: Methanol : Concentrated ammonia solution (100 : 1.5 v/v)

Methods

Solvent systems

Prepare developing solvent systems as accurately as possible by use of pipettes, dispensers and measuring cylinders. Leave the solvent system in the TLC tank for a time sufficient

to allow vapour phase saturation to be achieved prior to the analysis (with adsorbent paper-lined tanks, this takes approximately 5 minutes).

Preparation of standard solutions

These are all prepared at a concentration of 5 mg/ml in methanol and stored in a dark and cool place.

Sample solutions

Powder: Prepare a solution at a concentration of approximately 5 mg/ml in methanol.
Tablets: Grind a representative number of tablets (following sampling procedure) to a fine powder and prepare a solution as for powder.
Capsules: Remove the contents of a representative sample of capsules (following sampling procedure) and prepare a solution as for powder.

Spotting and developing

Apply as separate spots 1 µl and 5 µl aliquots of sample solution, 2 µl of the standard solution(s) and 2 µl of solvent (as a negative control) on the TLC plate. Spotting must be done carefully to avoid damaging the surface of the plate.

Analytical notes – TLC

- The starting point of the run i.e. the "spotting line" should be 2 cm from the bottom of the plate.
- The spacing between applications of sample (spotting points) should be at least 1 cm and spots should not be placed closer than 1.5 cm to the side edge of the plate.
- To avoid diffuse spots during development, the size of the sample spot should be as small as possible (2 mm).
- Allow spots to dry and place plate into solvent-saturated tank (saturation of the vapour phase is achieved by using solvent-saturated pads or filter paper as lining of the tank).
- Remove plate from the development tank as soon as possible as the solvent reaches the development line (10 cm from starting- line) marked beforehand; otherwise, diffused spots will occur.

Visualization/detection

Plates must be dried prior to visualization. The solvent can be allowed to evaporate at room temperature or removed with a hot air blower. It is important for proper colour development that all traces of diethylamine and ammonia be removed from the plate.

Visualization methods

A. UV light at 254 nm
Purple spots on an otherwise green-fluorescent plate are seen.

B. Acidified potassium iodoplatinate spray reagent
Dissolve 0.25 g platinic chloride and 5 g potassium iodide in water and make up to 100 ml; add 5 ml of concentrated hydrochloric acid to the resulting solution. Bluish purple spots on a pink background are observed when the plate is spayed with the reagent.

Interpretation

After visualization, mark spots (e.g. by pencil) and calculate retardation factor (Rf) values.

$$R_f = \frac{\text{Migration distance: from origin to centre of spot}}{\text{Development distance: from origin to solvent front}}$$

Table 2. Results (expressed as Rf x 100)*

Compound	Developing system	
	System A	System B
Methaqualone	40	74
Mecloqualone	30	74
Cocaine	52	67
Heroin	19	46
Diphenhydramine	56	55
Caffeine	5	63
Diazepam	29	75

Analytical notes

- Rf values are not always reproducible due to small changes in plate composition and activation, in solvent systems, tank saturation or development distance. Therefore, the Rf values provided are indications of the chromatographic behaviour of the substances listed.

*It is very common to express R_f as R_f x 100

- It is essential that reference standards be run simultaneously on the same plate.
- For identification purposes, both the Rf value and the colour of the spots after spraying with the appropriate visualization reagents should always be considered.

4.2.4 Gas chromatography (GC) with flame ionization detection (GC-FID)

The GC instrument of choice for routine analytical work is the narrow bore capillary gas chromatograph, using columns with internal diameter between 0.2 and 0.32 mm. However, in view of methods which are in use in a number of drug testing laboratories, non-capillary methods (i.e, IDs of 4 mm, methods B and C) for the analysis of methaqualone/mecloqualone are provided in this section.

Methods [5,6]

Preparation of internal standard solution

Dissolve tetraphenylethylene (or one of the n-alkanes) in dichloromethane to give a concentration of 4 mg/ml. Each analysis requires 5.0 ml.

Preparation of the standard solution

Accurately weigh 20 mg of methaqualone HCl (or mecloqualone HCl) standard and transfer quantitatively into a 10 ml Erlenmeyer flask. Accurately transfer 5.0 ml of internal standard solution to the flask followed by 2 ml of 1M sodium bicarbonate solution. Place the flask on a steam bath for 5-8 minutes, cool to room temperature, stopper and shake the flask. Allow the layers to separate and inject 1-2 µl of the dichloromethane layer (bottom layer) into the gas chromatograph.

Preparation of sample solution

Obtain a representative sample from the powder, tablets or capsules (refer to section 4.1 Sampling). Grind it to a fine powder. Accurately weigh a quantity of sample containing approximately 20 mg of methaqualone HCl (or mecloqualone HCl) and transfer quantitatively to a 10 ml Erlenmeyer flask.

Accurately transfer 5.0 ml of internal standard solution to the flask followed by 2 ml of 1M sodium bicarbonate solution. Place the flask on a steam bath for 5-8 minutes, cool to room temperature, stopper and shake the flask. Allow the layers separate and inject 1-2 µl of the dichloromethane layer (bottom layer) into the gas chromatograph.

The percentage of methaqualone HCl (or mecloqualone HCl) in the sample can be calculated using the general formula below:

$$Content\ (\%) = \frac{Wsamp_{calc}}{Wsamp_{nom}} \times 100$$

where

$Wsamp_{calc} = \frac{PAR_{sam}}{PAR_{std}} \times Wstd$ = calculated weight of analyte in the sample

$Wstd$ = calculated weight of analyte in the sample

$Wsamp_{nom}$ = nominal amount of sample used in preparation of the sample solution

$PAR_{std} = \frac{\text{Area of methaqulone peak in standard solution}}{\text{Area of internal standard peak in standard solution}}$ and

$PAR_{samp} = \frac{\text{Area of methaqulone peak in standard solution}}{\text{Area of internal standard peak in standard solution}}$

Method A—capillary column

GC operating conditions:

Detector:	FID
Column:	Fused silica, chemically bonded and cross-linked methyl silicone or methylphenylsilicone, such as OV-1, SE-54, BP-1, DB-1 or equivalent, 25 m, 0.25 mm ID, 0.25 µm film thickness
Carrier gas:	Nitrogen 1 ml/min
Split ratio:	20:1
Colum temp.:	250°C
Injector/detector temp:	275°C

Table 3. Results

Compound	DB-1 (retention indices)
Methaqualone	2135 (4.0 min)
Mecloqualone	2236 (4.8 min)
Caffeine	1796
Cocaine	2173
Diazepam	2431
Diphenhydramine	1900
Heroin	2577
Tetraphenylethylene	2442 (7.1 min)

Method B—packed column technique

GC operating conditions:

Detector:	FID
Column:	10% OV-101 on 100-120 mesh gas Chrom Q , 1.8 m, 4 mm ID glass
Carrier gas:	Nitrogen 60 ml/min
Column temp:	Initial temperature 150°C for 3 minutes; rate 20°C/min; final temperature 280°C
Injector/detector temp:	275°C/300°C

Method C—packed column technique

GC operating conditions:

Detector:	FID
Column:	3% OV-17 on 100-120 mesh gas Chrom Q, 1.8m, 4 mm ID glass
Carrier gas:	Nitrogen 60 ml/min
Column temp:	Initial temperature 150°C for 1 minute; rate 10°C/min; final temperature 280°C
Injector/detector temp:	275°C/300°C

Table 4. Results (Methods B and C) [7]

Compound	Retention time (min)	
	10% OV-101	3% OV-17
Anthranilic acid	5.5	2.9
N-acetylanthranilic acid	8.2	5.3
o-toluidine	1.7	0.7
o-chloroaniline	2.2	0.9
Methaqualone	12.8	10.5
Mecloqualone	13.7	11.5
o-methyl acetanilide	5.7	3.1
o-chloro acetanilide	5.4	2.5
2-methyl-3-*o*-carboxyphenyl-4-quinazolinone	17.6	14.7

4.2.5 Gas chromatography–mass spectrometry (GC-MS)

GC-MS is one of the most commonly used techniques for the identification of forensic drug samples. As a hyphenated technique, it unifies the separation power and sensitivity of a GC with the analyte specificity of a spectroscopic technique. It can provide high specific spectral data on individual compounds in a complex mixture without prior isolation.

Sample preparation and extraction procedure

Samples are pulverized and homogenized with a mortar and pestle. A suitable quantity (1 g) of material is transferred into a test tube (10 ml). Chloroform (5 ml) is added and the mixture vortexed for 10 seconds. The supernatant is filtered through anhydrous sodium sulfate, an aliquot of the supernatant (1 µl) is then transferred to a sample vial (2 ml). The sample is reduced to dryness under a stream of nitrogen and the residue reconstituted in chloroform (1 ml) prior to GC/MS analysis.

Preparation of standard solutions

Prepare a standard solution of methaqualone at a concentration of 100 mg/ml in chloroform.

GC-MS operating conditions [8]

GC oven conditions:	Column temperature initially set at 100°C and ramped immediately after injection to 280°C at a rate of 10°C/min with a final isotherm of 5 minutes.
Column:	DB-5MS, HP-5MS , 30 m x 250 µm; df 0.25 µm
Inlet:	Mode: splitless (splitter vented 1.5 min); Temperature = 250°C
	Carrier gas: helium, 1ml/min, constant flow.
Detector:	Ionization mode: EI mode, 70 eV; Transfer line temp: 280°C; Ion source temperature: 230°C
MS parameters:	Solvent delay 4.00 minutes; Scan parameters: TIC, scan range: 40 – 450 m/z

Identification is accomplished by comparing the retention time and mass spectrum of the analyte with that of a reference standard. All compounds identified by GC-MS and reported by the analyst must be compared to a current mass spectrum of the appropriate reference standard, preferably obtained from the same instrument, operated under the same conditions. Commercial mass spectral libraries or user generated spectra should be used for reference purposes only. Quantification can be done by using IS method (refer to 4.2.4).

The principal ions in the mass spectrum of methaqualone are m/z 235, 250, 91, 233, 236, 65, 76, 132

Table 5. Results of GC-MS analysis

Compound	Retention time (min)
Methaqualone	12.2
Diphenhydramine	15.2
Cocaine	15.1
Diazepam	17.2
N-Acetylanthranilic acid	10.5
Anthranilic acid	6.8

Compound	Retention time (min)
Acetanthranil	6.5
o-methyl acetanilide	7.4
o-toluidine	5.7

4.2.6 High performance liquid chromatography (HPLC)

All materials for the sample and standard solutions are prepared in the mobile phase to produce final concentrations of approximately 1 mg/ml. Quantitation is achieved by the external standard method using analyte peak areas (refer to section 4.2.4 Gas chromatography (GC) with flame ionization detection GC-FID).

Chromatographic conditions [9]

Column:	ODS Hypersil (or equivalent), 250 mm x 4.6 mm ID
Mobile phase:	Acetonitrile: 1% ammonium acetate solution : 2.5% aqueous diethylamine (40:45:15 by volume; adjust pH to 8-9 units by addition of ammonia or acetic acid)
Flow rate:	1.5 ml/min
Detection:	UV at 254 nm
Injection volume:	5 µl

Table 6. Results of HPLC analysis

Compound	Capacity factor (k)
Methaqualone	5.1
Mecloqualone	6.1
Caffeine	1.1
Cocaine	7.9
Diazepam	9.9
Diphenhydramine	13.8
Heroin	4.3

4.2.7 Fourier transform infrared (FTIR) spectroscopy

The confirmation of the identity of a substance can be achieved by FTIR. Unequivocal identification of methaqualone and mecloqualone is thus possible from each unique spectrum. For powders, considered from prior chromatographic analysis to be reasonably pure, the infrared spectrum of the powder may be run directly in a KBr disc for comparison with those of methaqualone or mecloqualone free bases or HCl salts. For tablets, capsules and mixture of powders, an extraction procedure would be required to liberate the free base in a pure form.

Analytical notes

- The KBr disc method consists of grinding a dry sample to a very fine powder, then mixing about 1 mg of homogenized sample powder with 200 mg of carefully dried and ground KBr. After grinding, the mixture is pressed into a thin transparent disk.
- KBr should be "IR Grade" and dried at 110°C for a minimum of one hour. It can be stored in a dessicator containing a strong desiccant (silica gel) or left in the oven and removed when required

Result [5, 10]

Methaqualone: Principal peaks at wave numbers: 1682, 1599, 1565, 770, 1265, 697 (KBr disk)

Mecloqualone: Principal peaks at wave numbers: 1682, 1605, 768, 782, 1282, 1583 (KBr disk)

The IR spectra of methaqualone and mecloqualone are quite similar except for the "fingerprint" region from 1300 to 625 cm-1. Mecloqualone can be recognized by its strong absorption at 1275.

4.2.8 Ultraviolet (UV) spectrophotometry

Methaqualone and mecloqualone show the following absorption peaks in aqueous acid and alkaline media.

Aqueous acid: 234 nm (A (1% 1 cm) = 1320), 269 nm.

Aqueous alkali: 265 nm (A (1% 1 cm) = 347), 306 nm.

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