Mediterranean Journal of Pharmacy & Pharmaceutical Sciences
www.medjpps.com

ISSN: 2789-1895 online
ISSN: 2958-3101 print

ORIGINAL RESEARCH article

Rapid roadside drug testing in saliva using *in vitro* tongue

Mohamed H. Assaleh [1 *], Shaban G. Elosta [2], Ravi Shankar Ayyanar [3] Prakash Katakam [4] and **Shanta K. Adiki [5]**

[1] CID Forensics Laboratory, Toxicology Unit Lab., Tripoli, Libya, [2] Department of Chemistry, Faculty of Science, University of Zawia, Libya, [3] Additional Director General of Police, Law and Order and Road Safety, Andhra Pradesh State Police, Mangalagiri - 522503, Andhra Pradesh, India, [4] Indira College of Pharmacy, Nanded 431606, Maharashtra, [5] Rajarshi Shahu College of Pharmacy, Markhel 431718, Nanded, Maharashtra, India
[*]Author to whom correspondence should be addressed

Received: 01-10-2022, **Revised:** 27-10-2022, **Accepted:** 05-11-2022, **Published:** 31-12-2022

HOW TO CITE THIS
Assaleh et al. (2022) Rapid roadside drug testing in saliva using in vitro tongue.
Mediterr J Pharm Pharm Sci. 2 (4): 13-24. [Article number: 85]. https://doi.org/10.5281/zenodo.7479700

Keywords: Chemical spot test, colorimetric tests, drug test, *in vitro* tongue, roadside testing

Abstract: The propagation of drugs on the road was the main reason for focusing on the development of the rapid methods which are used to detect the presence of drugs which may have been taken. Oral fluid has become a popular specimen to test for the presence of drugs. *In vitro* tongues were prepared with different concentrations of hydroxypropyl methylcellulose (HPMC E4M); one of these concentrations was chosen to be used for tests. Chemical reagents were prepared which included: cobalt thiocyanate, fast blue B test, Marquis, Mandelin and Zimmerman reagents which were used to examine eight drugs in three different concentrations, each of 10.0 mg/mL, 5.0 mg/mL and 1.0 mg/mL where each reagent was used for a particular drug. The drugs were amphetamine, cannabis, cocaine, 3,4-methylenedioxymethamphetamine (MDMA), codeine, diazepam, heroin, methadone and morphine. Each drug was tested by a specific reagent. The difference in the concentrations gave various results in terms of achieving positive results and the ratio of the clarity of the colour. The amount of the drug on the tongue was between 20 µg and 400 µg. Positive and negative results were obtained in this study. Most of the high concentrations gave positive results; however, the low concentrations gave different results which were between positive, negative and light or very light in colour. In conclusion, there is an explanation of the difference in sensitivity of the effects of the different kinds of reagents in the drug, such as cobalt thiocyanate which was more sensitive at the low concentration of heroin and gave a clear result.

Introduction

Drunk and drugged driving are a serious problem all over the world. This has significant implications for road safety because drunk or drugged driving may increase a driver's chances of being involved in a car accident as compared to a drug-free driver. Recent research has modelled the relationships between the prevalence of tetrahydrocannabinol (THC) and methamphetamine in fatally and seriously injured drivers. An increase in targeted and random roadside drug tests can save a significant number of fatal crashes and serious injury crashes every year [1]. Many mobile roadside drug testing devices have recently been introduced to the market as the number of drug-impaired drivers involved in crashes has increased. Oral fluid, urine or blood matrices

ISSN: 2789-1895 online
ISSN: 2958-3101 print

are used in these devices. During the past ten years, researchers have considered oral fluids a useful biological matrix, specifically as an alternative to blood [2]. When looking for reasonably non-invasive ways to identify relatively recent drug use, the use of oral fluid has been found to hold a lot of potential. There seems to be a decent correlation between the medication concentrations in blood and oral fluid, despite the fact that there are a number of variables that can impact this concentration. Collection methods have the potential to artificially alter oral fluid output and its resulting pH. To ensure appropriate stability and recovery of ingested medicine, it is crucial to inspect devices used to collect oral fluid [3].

The purpose of rapid roadside drug testing is to determine the presence of drugs that may have been taken. Until now, there has not been a roadside drug test that can determine the range of possible drugs that a driver might have consumed. Police have used many tests to measure body coordination, such as walking in a straight line, standing on one leg, or touching their nose need [4]. Many road accidents have happened under the influence of drugs; roadside drug testing includes body fluids such as saliva, urine, sweat, breath and tests on other body fluids [5]. Due to these abnormal behavior of drivers under the influence of drugs, law enforcement agencies have been putting greater emphasis on controlling driving under the influence of drugs. Driving under the influence is also a big problem in the EU, and USA [6]. Further, it was found that 28.0% to 53.0% of the drivers who have been seriously injured in accidents were under the influence of a psychoactive drug (mostly alcohol, medicinal or recreational drugs [7]. The possibility of having a saliva specimen is the best advantage of having a saliva specimen on roadside testing which can be collected so easily [8]. On-site, oral fluid specimens are performed for a quick test. If a positive result is obtained, an oral fluid or blood specimen is collected and sent to laboratories for the confirmation test [9]. Law enforcement agencies most commonly use oral fluid to detect the presence of illicit drugs in drivers. With established method detection procedures and devices in place for alcohol, analytical chemists and other scientists are focusing their efforts on establishing analytical cut-offs and devices for the detection of drugs at the roadside. With many commercially available kits on the market and none meeting the required standards at this time, a search for alternative methods is ongoing. Because of the advantage of drug testing in oral fluid in cases of driving under the influence, it has increased, especially in recent years. It is easy to collect by non-medical personnel without embarrassment and the correlation is better between impairment and the presence of drugs in oral fluid. Since the 1980s, many surveys have been performed using saliva and researchers have encountered some problems which are related to insufficient sample volumes and the sensitivity of analytical methods. Stable progress was shown in the collection of samples and the knowledge of toxicity in oral fluid confirms the results in toxicology [5]. Several methods have been used to collect oral fluids by a variety of techniques which include simple expectoration into a plastic tube or using an absorbent material such as cotton, fiber wad or foam pad in the oral cavity to absorb oral fluid directly. The responsibility is for a toxicologist and a laboratory professional to prove if the specimens are positive or negative [10].

One of the devices that are used to detect illegal drugs in sweat, saliva, or on the surface of the skin is the drug wipe, which is a pen-size detector, drug wipes are available in single, twin and five-panel configurations [11, 12]. This device is available for the detection of opiates, cocaine, amphetamine, methamphetamine, ecstasy and cannabinoids [11, 13]. The process takes only seconds. However, if the result is positive, there is no oral fluid for any confirmatory assay. Other on-site drug detection devices include Orasure Uplink®, Drugread®, CozartRapiscan®, CozartRapiScan®, DrugTest®, OralScreen® and SalivaScreen® [8, 14]. Chemical spot tests are described as sometimes referred to as presumptive tests or color tests. Several color tests involving a number of different substances are available to the drug chemist for presumptive test purposes [15]. As one of the earliest test methods used by criminalists and toxicologists for the presumptive identification of poisons and drugs. The popularity of these tests is due to several reasons: Firstly, they use simple chemical reactions that lead to visible results that can be interpreted by the naked eye.

ISSN: 2789-1895 online
ISSN: 2958-3101 print

Table 1: Colorimetric tests used for identification of drug

Drug		Reagent	Color	Reference
Amphetamine	B	Marquis reagent	Orange	[15, 18, 19]
		Mandelin	Green	[20]
Anabolic steroids	C	Sulfuric acid ethanol	Fluorescent derivative	[18]
		Zimmerman test	including yellow-orange and	[48]
			pink violet	[20]
			Blue	
Benzodiazepines (Diazepam and Oxazepam)	C	Zimmerman test	Reddish purple	[18, 19]
		Formaldehyde sulfuric acid	Orange	[21]
Cannabis `	B	Duquenois reagent	Purple/Violet-blue	[18, 21]
		Fast blue B	Pink	[18]
		p-dimethylamino benzaldehyde	Red/violet	[21]
Cocaine	A	Cobalt thiocynate reagent	Blue	[15, 18, 22]
		p-Dimethylamino	(100 ºC for 3 min) Red	[21]
		benzaldehyde		[21]
Codeine	B	Marquis reagent	Violet	[21, 22]
		Liebermann's test	Black	[21]
		Mandelin reagent	Green	[21, 22]
Dihydrocodeine		Marquis reagent	Purple	[18, 21]
	B	Mandelin reagent	Grey-green	[21]
Dipipanone	A	Mandelin reagent	Green then blue	[18, 21]
Ecstasy/ MDMA	A	Mandelin reagent	Dark purple	[18]
		Marquis reagent	Dark purple	[18, 20, 21]
		Meckere agent	Dark purple	[18]
		Simom's reagent	Dark blue	[18, 22]
Heroin/ Diamorphine	A	Marquis reagent	Purple	[18, 19, 21]
		Liebermann'stest	Black	[19, 21]
		Mandelin reagent	Blue-grey	[21]
		Cobalt thiocynate reagent	Blue	[15]
		Froehdes reagent	Purple	[19]
LSD/ Lysergic acid diethylamide.	A	Marquis reagent	Olive black	[18, 21]
		Van Urk's reagent	Purple	[18]
		p-Dimethylamino	Violet	[21]
		benzaldehyde	Purple	[19]
		Erlich reagent	Fluorescence	[19]
		UV light		
Methadone	A	Marquis Reagent	Brown	[18]
		Liebermann's test	Brown-orange	[21]
		Mandelin reagent	Green-blue	[21, 22]
Methylphenidate	B	Marquis reagent	Orange	[18]
		Liebermann's test	Orange	[21]
Morphine	A	Marquis reagent	Violet/purple	[18, 19, 21]
		Ferric chloride	Blue	[21]
		Liebermann's test	Black	[19, 21]
		Mandelin reagent	Blue-grey	[21]
Pethidine	A	Marquis reagent	Orange	[18]
		Liebermann's test	Red-orange	[21]

Secondly, the laboratory materials and reagents that are needed to carry out the tests are easily available and inexpensive. Thirdly, it is easy to use by technicians without extensive training. Fourthly, the tests require very little reagents and materials and fifthly, law enforcement agents can apply it in the field. These tests are

ISSN: 2789-1895 online
ISSN: 2958-3101 print

still an integral part of the forensic laboratory [16]. Color spot tests are usually the quickest and simplest chemical tests that can be applied to a sample. Most color spot tests are quite sensitive; thus, successful tests need only a small quantity of samples to be completed [17]. They are only presumptive and give an indication of the possible presence of drugs, so they must be confirmed by other tests. Color tests have several advantages, such as they are inexpensive, rapid and may be used by unskilled operators in the field, but it is best to confirm the test in the laboratory. Applications of color tests are most useful in detecting scene residues, pharmaceuticals and to a lesser extent, biological fluids such as urine and stomach contents. Moreover, color tests are important in clinical toxicology, particularly for patients in accident and emergency cases. In cases of some symptoms of poisoning, clinicians have to know as soon as possible what substances they are dealing with before they can start treatment. In these circumstances, color tests are considered a good indication for drug compounds of mixed classes and they give faster results than chromatography techniques and immunoassays [18]. In **Table 1** the classification of drugs, color tests and the colors that appeared from the tests are presented.

Materials and methods

Cannabis from West Yorkshire Police, cocaine from Lloyd's pharmacy, heroin from West Yorkshire Police, codeine (LGC standard as codeine sold), amphetamine from West Yorkshire police, diazepam from Lloyd's pharmacy, methadone as LGC standard and another methadone from Lloyds Pharmacy, 3,4-methylene-dioxymethamphetamine (MDMA) as LGC standard, morphine as LGC standard, pH 7 phosphate buffer tablets from Fisher Scientific were used. Different reagents are available for the wide range of color tests of drugs; these are just presumptive tests for initial results. The following shows details of such reagents and the methods of preparation which are most frequently used:

Cobalt thiocyanate reagent: Dissolved 2.0 g of cobalt (II) thiocyanate [22].

Duquenois - Levine reagent, modified: The steps following are to prepare this reagent by preparing two solutions: Solution A was prepared by adding 2.0 g of vanillin and 2.5 ml of acetaldehyde to 100 ml of 95.0% ethanol. Solution B was composed of concentrated hydrochloric acid and solution C was chloroform. The reagent was prepared by adding one volume of solution A to the drug and mixing non-vigorously for a minute. After that, one volume of solution B and agitated gently until a definite color was produced. Next, they added three volumes of solution C and observed if the color was extracted from the mixture to A and B [15].

Fast Blue B: 1.0 g of fast blue B salt (diazotised o-dianisidine) was dissolved in 50 mL of distilled water and two drops of concentrated hydrochloric acid were added [20].

Ferric chloride: Dissolved 3.3 g of ferric chloride hexahydrate or 2.0 g of anhydrous ferric chloride in 100 ml of distilled water [16].

Froehde reagent: Dissolved one gram of sodium molybdate or molybdic acid in 100 ml of hot concentrated sulfuric acid [20].

Liebermann's reagent: Added one gram of potassium or sodium nitrite to 10 ml of concentrated sulfuric acid with swirling and cooling to absorb the brown fumes [20].

Mandelin reagent: Carefully dissolved one gram of ammonium vanadate in 100 ml of concentrated sulfuric acid [16].

Marquis reagent: To prepare the reagent, carefully add 100 ml of concentrated sulfuric acid to 1 ml of 40.0% (v/v) formaldehyde [18].

Mecke reagent: In 100 ml of concentrated sulfuric acid, dissolved 0.5 g of selenious acid [16].

ISSN: 2789-1895 online
ISSN: 2958-3101 print

Para-dimethylaminobenzaldehyde: Dissolved 2.0 g para-dimethylaminobenzaldehyde in 50.0 ml of concentrated hydrochloride and 50 ml of 95.0% ethanol [22].

Simom's reagent: Solution A was prepared by dissolving one gram of sodium nitroprusside in 50 ml of distilled water, and then adding 2.0 ml of acetaldehyde to the solution. Solution B was prepared by dissolving 2.0% sodium carbonate in distilled water. Then the drug was added to one volume of solution A, followed by two volumes of solution B [22].

Sulfuric acid ethanol: Gradually added 10 ml of sulfuric acid to 90 ml of ethanol [20].

Zimmermann reagent: In 500 ml of water, 70 g of $MnSO_4.4H_2O$ was dissolved 125 ml of concentrated sulfuric acid and 125 ml of 85.0% H_3PO_4, and diluted to one liter [19]. Moreover, this reagent consists of two component solutions, 1.0% 2,4-dinitrobenzene in methanol (1) and 15.0% aqueous KOH (2). To perform this test, a few drops of component (1) followed by a few drops of component (2) were added directly to the test substrate [15]. A nitric acid test is used to distinguish between morphine and heroin [23]. Moreover, nitric acid makes it possible to separate morphine (orange-red color), codeine (orange color) and heroin (yellow color) [24].

Preparation of HPMC films (in vitro tongue): The plan of the practical work was to prepare different concentrations of 1, 2, 4 and 8 g HPMC-E4M on an *in vitro* tongue in order to decide which concentration would be most useful to apply the color tests for drugs. First of all, a 1.0% concentration of HPMC was prepared by adding 2.0 ml of propylene glycol to 1.0 g of HPMC and stirring it well using a pestle and mortar and then carefully and slowly adding 98 ml of cold distilled water whilst stirring. Another set of concentrations of 2.0%, 4.0% and 8.0% of HPMC was prepared by adding 2.0 ml of propylene glycol to 2, 4 and 8 g of HPMC and treated in a similar way to the first concentration. Both 1.0% and 2.0% concentrations were left in the refrigerator for 2 hrs.., then carefully spread onto the surface of the microscope slide using a wooden stick and dried with a hair dryer; three further layers were added after drying each time to increase the thickness of the layer. However, the concentrations of 4.0% and 8.0% were left in the refrigerator for four days because it took about 2 hrs. of stirring which led to a lot of bubbles being created in the product.

In vitro tongue selection: An *in vitro* tongue was chosen because it is an important method for conducting presumptive tests for drug detection in the laboratory.

Results

Different concentrations were prepared in order to choose the best one **(Figure 1)**. Five slides were prepared from each concentration, as shown in **Figure 1.** The *in vitro* tongues were compared with each other. The *in vitro* tongues, which contained a layer of 1.0% of HPMC have the specifications that make them eligible to be used to test the appearance of drugs using color spot tests. The most important specifications chosen were transparency, being smooth to the touch and having no air bubbles. On the other hand, the *in vitro* tongues, which contained a layer of concentration of 2.0% of HPMC, as shown in **Figure 1**, had some air bubbles which made them unclear and for this reason, this concentration was excluded. In addition, the concentrations of 4.0% and 8.0% had a lot of bubbles or a white layer on the surface which led to these being excluded. The 1.0% layer of concentration was placed on about thirty slides, three different times, and dried with a hair dryer each time. Moreover, the slides were weighed before and after adding the layer using wood splints.

Table 2 shows the weight of the HPMC on the slides (the weight of the layer was chosen for the name of the column). The drugs were dissolved in methanol; the concentrations of 1.0, 5.0 and 10.0 mg/ml were prepared for most of them to start the color test of these drugs on the *in vitro* tongue, which had also been prepared. Firstly, 20.0 µl of pH 7 buffer solution was added to the *in vitro* tongue. Next, 20, 35, or 40 µl of drugs were

ISSN: 2789-1895 online
ISSN: 2958-3101 print

added to the *in vitro* tongue and swabbed with filter paper. Finally, the reagent was dropped onto the filter paper using a dropper or spray. Some of the tests were positive and some of them were negative; the results that were obtained are given in **Table 2**.

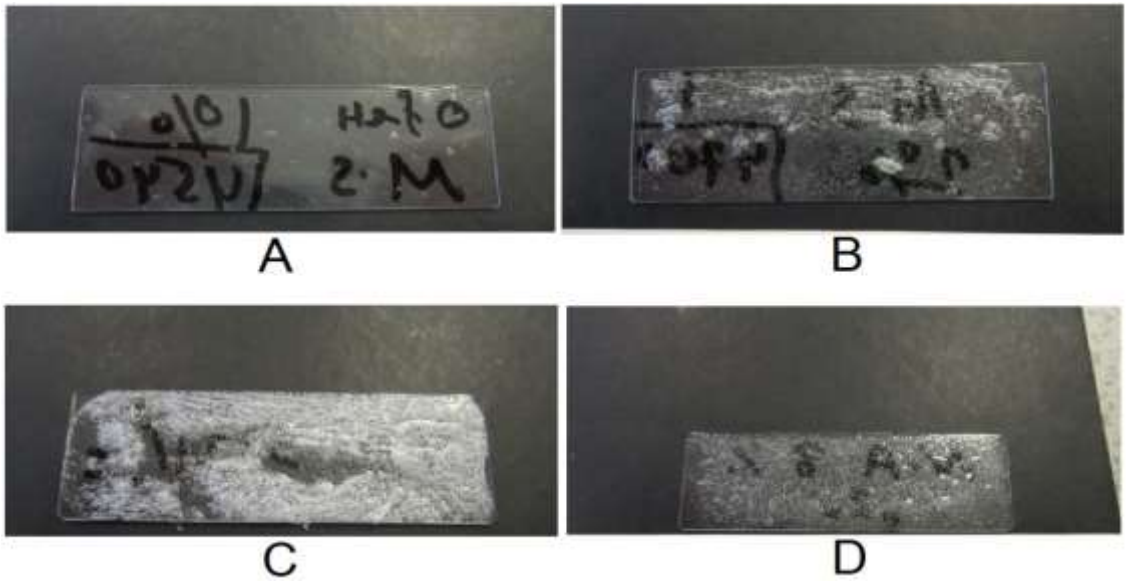

Figure 1: *In vitro* tongues prepared with A (01.0%), B (2.0%), C (4.0%) and D (8.0%) of HPMC E4M

Table 2: Amount of the drug placed onto the *in vitro* tongue in each concentration and volume

Concentration mg/ml	Volume (µl)	Amount on the tongue (µg)
10	20	200
10	40	400
05	20	100
05	40	200
01	20	020
01	35	035
01	40	040

The buffer solution was chosen based on a value of pH of saliva between 6.2 and 7.4. The litmus paper test was used to test the pH of saliva and buffer solution as in **Figure 2**. The pH of saliva and pH 7.0 buffer solution indicate the similarity of using saliva and buffer solution.

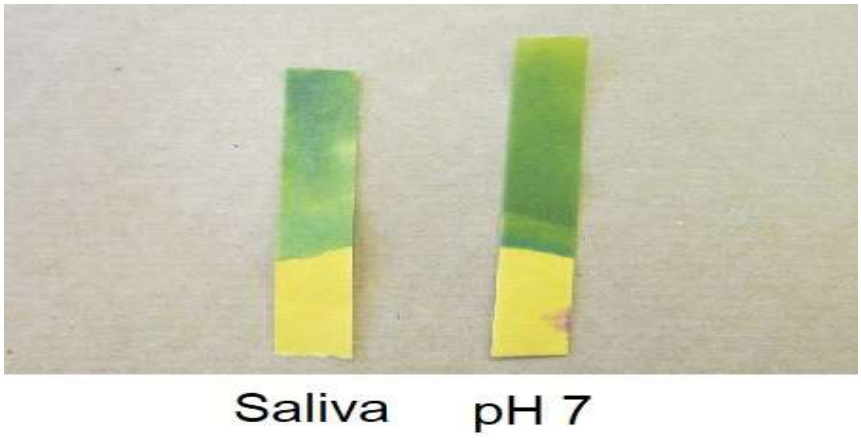

Figure 2: pH of saliva and pH 7 buffer solution

ISSN: 2789-1895 online
ISSN: 2958-3101 print

The calculation of the amount of the drug onto the *in vitro* tongue with a concentration 10.0 mg/ml and volume 20.0 µl: The concentration of the drug is 10.0 mg/ml ≈ 10 × 103 µg / 1 × 103 µl.

$$(10 \times 10^3\ \mu g) \rightarrow (1 \times 10^3\ \mu l)$$
$$X \rightarrow (20\ \mu l)$$
$$X = (10 \times 10^3\ \mu g)\ (20 \mu l) / (1 \times 10^3\ \mu l) = 200\ \mu g$$

By following the same steps as the previous calculation, the amount of the drugs on the *in vitro* tongues was shown in **Table 2**. The first drug that was tested was amphetamine, with a concentration of 10 mg/ml. A pipette was used to add 20.0 µl of pH 7.0 buffer solution to the in vitro tongue. The next 20 µl (need concentration in 20 µl) of amphetamine was also added to the *in vitro* tongue and swabbed with filter paper; Marquis reagent was then dropped onto the filter paper using a dropper, and the result was negative. Furthermore, the amount of amphetamine was increased to 40 µl containing 10 mg/ml to the other slide and swabbed filter paper, then tested by Marquis reagent, which gave a negative result as well. The second drug was cannabis, which was tested in three concentrations: 1.0, 5.0 and 10.0 mg/ml. The first color test was with a concentration of 10 mg/ml. A 20 µl of pH 7 buffer and 20 µl of cocaine were added to the *in vitro* tongue by pipette and swabbed with filter paper. The ratio of the drug in the *in vitro* tongue after calculation was 200 µg. The fast blue B reagent was sprayed onto the filter paper. A pink color resulted in the filter paper. **Figure 3** shows the color which was obtained. The second concentration was at 5.0 mg/ml of cannabis. The same previous test steps were applied to the *in vitro* tongue. The filter paper was tested by the fast blue B reagent; a pink color was obtained, which was slightly less than with the concentration of 10 mg/ml, because the amount of the cannabis on the *in vitro* tongue was 100 µl as shown in **Figure 3**. The third concentration was 1.0 mg/ml where a very light pink color was obtained after testing the drug because of the small percentage of the drug on the *in vitro* tongue, which was 20 µg after being calculated. **Figure 3** shows the cannabis color test and its results.

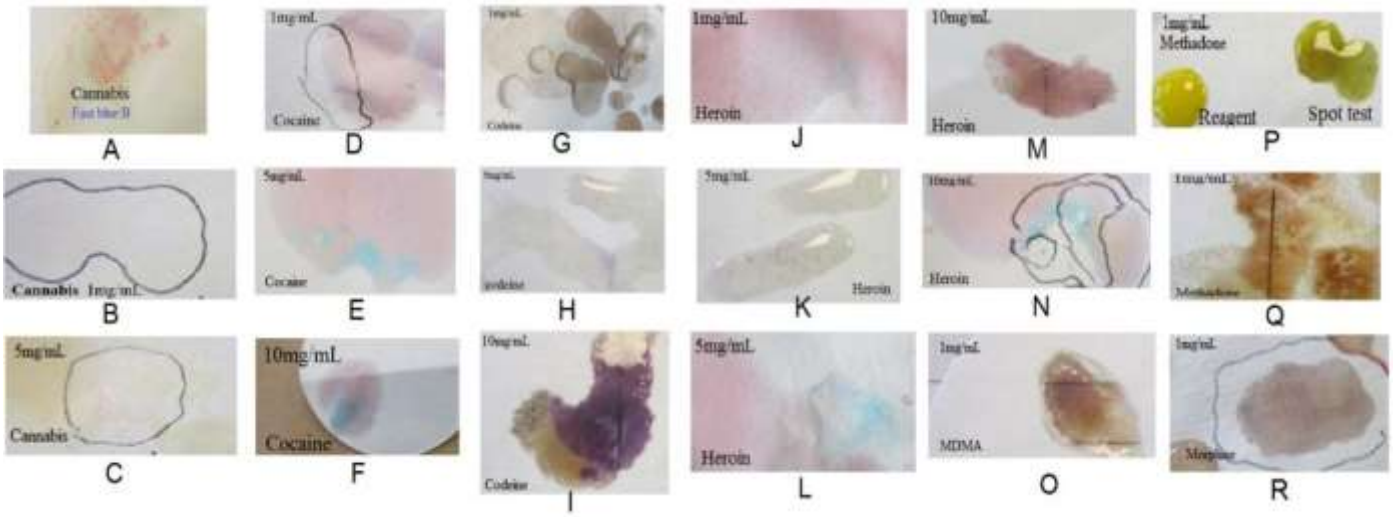

Figure 3: Findings of the colour tests for (A) 10 mg/ml and 20 µl of cannabis, (C) 5 mg/ml and 20 µl of cannabis, (C) 5 mg/ml and 20 µl of cannabis, (D) 1 mg/ml and 20 µl of cocaine (E) 10 mg/ml and 20 µl of cocaine, (F) 10 mg/ml and 20 µl of cocaine, (G) 1 mg/ml and 35 µL of codeine, (H) 5 mg/ml and 20 µl of codeine, (I) 10 mg/ml and 20 µl of codeine, (J) 1 mg/ml and 20 µl of heroin (cobalt thiocyanate reagent), (K) 5 mg/ml and 20 µl of heroin (Marquis reagent), (L) 5 mg/ml and 20 µl of heroin (cobalt thiocyanate reagent), (M) 10 mg/ml and 20 µl of heroin (Marquis reagent), (N) 10 mg/ml and 20 µl of heroin (cobalt thiocyanate reagent), (O) 1 mg/ml and 20 µl of MDMA, (P) 1 mg/ml and 20 µl of methadone, (Q) 1 mg/ml and 35 µl of methadone, and (R) 1 mg/ml and 40 µl of morphine.

ISSN: 2789-1895 online
ISSN: 2958-3101 print

The third drug that was tested was cocaine; the first drug test was with a concentration of 10 mg/ml. By pipette, 20 µl of pH 7.0 was added to the *in vitro* tongue, which was then swabbed with filter paper. A drop of cobalt thiocyanate reagent was dropped onto the filter paper, which gave a positive result, and a blue color appeared on the dropper. The amount of the drug on the *in vitro* tongue which was calculated was 200 µg. The second concentration of cocaine that was tested was 5.0 mg/ml; the same previous steps were followed. The test was positive and a blue color resulted when the cobalt thiocyanate reagent was dropped. The color was light and the ratio of cocaine was calculated on the *in vitro* tongue and found to be 100 µg. The third concentration of cocaine was one mg/ml; after adding 20 µl of buffer solution and 20 µl of cocaine into the *in vitro* tongue and taking a swab using filter paper, the cobalt thiocyanate was dropped, the result was positive, and a very light blue color resulted; it was very small spot because the amount of the drug in the *in vitro* tongue was 20 µg. The fourth drug that was tested was codeine; the first color test was for a concentration of 10 mg/ml where, 20 µL of buffer solution pH 7.0 and 20 µl of codeine were added to the *in vitro* tongue, which was then swabbed using filter paper. A drop of Marquis reagent was dropped onto the filter paper, which gave a positive result. A dark purple color resulted, as shown in **Figure 3**. In the experiment, 200 µg of codeine was used. The second concentration was 5 mg/ml; the same steps of the color test were followed in the *in vitro* tongue. By adding cobalt thiocyanate reagent, a light purple color was created as shown in **Figure 3**. The amount of codeine on the *in vitro* tongue after calculation was 100 µg. The third color test for codeine used a concentration of one mg/ml; 20 µl of buffer solution pH 7 and 20 µl of codeine were added into the in vitro tongue and swabbed with filter paper before dropping Marquis reagent on the filter paper. The result was negative, no color was detected. In this test, the amount of cocaine in the *in vitro* tongue after calculation was 20 µg. Further, the amount of codeine was increased to 35 µl in another *in vitro* tongue and tested again by Marquis reagent, which obtained a very light purple color. The amount of codeine in the *in vitro* tongue was calculated and found to be 35 µg. The fifth drug tested was diazepam, with a concentration of 5 mg/ml in 20 µl of buffer solution and both 20 and 40 µl in separate *in vitro* tongues. They were swabbed using filter paper. The Zimmerman reagent was dropped into each *in vitro* tongue which gave negative results. The Zimmerman reagent was prepared twice with the same negative results.

The sixth drug tested was heroin. The first concentration was 10 mg/ml; 20 µl of buffer solution and 20 µl of heroin were added to the two *in vitro* tongues and swabbed using filter paper. The Marquis reagent was dropped on one of the filter papers and resulted in a purple color, as shown in **Figure 3**. When cobalt thiocyanate reagent was dropped into the other one, a blue color resulted. The amount of heroin in the *in vitro* tongue was 200 µg. The second concentration of heroin that was tested was 5.0 mg/ml; the same steps as previously were followed with the Marquis reagent and cobalt thiocyanate. The result was positive. A very light purple color was obtained with Marquis reagent and a blue color was obtained with cobalt thiocyanate reagent. Cobalt thiocyanate reagent was more sensitive to heroin than the Marquis reagent. The third concentration of heroin tested was 1.0 mg/ml; the same steps were followed. The result with the Marquis reagent was negative. However, a very light blue color was obtained with cobalt thiocyanate reagent.

The seventh drug tested was methadone: both standard methadone from LGC and methadone from a pharmacy; 20 µl of buffer solution and 20 µl of one mg/ml methadone (LGC standard) were added to the *in vitro* tongue, and then swabbed using filter paper. A green color was obtained when Mandelin reagent was dropped into the *in vitro* tongue. The second test was for 1.0 mg/ml of methadone from a pharmacy by adding 20 µl of buffer solution and adding 20 µl and 35 µl of methadone into the separated *in vitro* tongues and swabbing with filter paper. The Marquis reagent was dropped into the filter paper; the 20 µl of methadone that was added gave a negative result; however, the 35 µl of methadone *in vitro* tongue that was added gave a positive result. A brown color was obtained. The eighth drug tested was 1.0 mg/ml of MDMA. By following the same steps, by adding 20 µl of buffer solution and 20 µl of the drug, Marquis reagent was used and gave

ISSN: 2789-1895 online
ISSN: 2958-3101 print

a positive result. The purple color was obtained as shown in **Figure 3**. The final drug tested was 1.0 mg/ml morphine; two separate amounts were taken in two *in vitro* tongues which were 20 and 40 µl and they were tested with Marquis reagent. The spot test of 20 µl was negative; however, the spot test of 40 µl was positive, light purple color was obtained as shown in **Figure 3**. The findings as summarized in **Table 3**, the following observations were noted: amphetamine gave negative results with Marquis reagent, even when the amount of amphetamine was increased to 40 µl, although it gave good results with other drugs. Cobalt thiocyanate reagent was successful with cocaine, which gave a clear blue color with concentrations of 10 and 5.0 mg/ml. The Marquis reagent gave positive results with concentrations of 10 and 5.0 mg/ml of codeine; the amounts of the drug on the *in vitro* tongue were 200 and 100 µg. However, it gave a negative result with concentration of 1.0 mg per ml, which was the amount of the drug on the *in vitro* tongue (20 µg). The Zimmerman reagent gave a negative a concentration of 5.0 mg/ml of diazepam and a volume of 20 and 40 µl of the drug, which means that the amounts of the drug on the *in vitro* tongue were 100 and 200 µg of diazepam, despite the fact that the reagent was prepared twice to exclude doubts.

Table 3: Findings of colour tests

No.	Drug	Weight of HPMC layer (g)	Sample Concentration (mg/ml)	Volume (µl)	Weight (µg)	Reagent	Colour
1	Amphetamine	0.0557	10	20	200	Marquis	No colour
2	Amphetamine	0.0754	10	40	400	Marquis	No colour
3	Cannabis	0.1023	10	20	200	Fast blue B	Pink
4	Cannabis	0.0824	5	20	100	Fast blue B	Pink
5	Cannabis	0.0798	1	20	20	Fast blue B	Very light pink
6	Cocaine	0.0980	10	20	200	Cobalt thiocyanate	Blue
7	Cocaine	0.0931	5	20	100	Cobalt thiocyante	Blue
8	Cocaine	0.0934	1	20	20	Cobalt thiocyanate	Very light blue
9	Codeine	0.0455	10	20	200	Marquis	Dark purple
10	Codeine	0.0504	5	20	100	Marquis	Purple
11	Codeine	0.0803	1	20	20	Marquis	No colour
12	Codeine	0.0491	1	35	35	Marquis	Very slight purple
13	Diazepam	0.0714	5	20	100	Zimmerman	No colour
14	Diazepam	0.0398	5	40	200	Zimmerman	No colour
15	Heroin	0.0788	10	20	200	Marquis Cobalt thiocyanate	Purple Blue
16	Heroin	0.1109	5	20	100	Marquis Cobalt thiocyanate	Very light purple Blue
17	Heroin	0.0329	1	20	20	Marquis Cobalt thiocyanate	No colour Light blue
18	Methadone LGC	0.0689	1	20	20	Mandelin	green
19	Methadone "Pharmacy"	0.0783	1	20	20	Marquis	No reaction
20	Methadone "Pharmacy	0.0589	1	35	35	Marquis	Light brown
21	MDMA LGC St	0.0909	1	20	20	Marquis	Purple
22	Morphine	0.0512	1	20	20	Marquis	No colour
23	Morphine	0.0644	1	40	40	Marquis	Light purple

The weight of HPMC layer' is the amount of HPMC layer on the surface of the microscope slides.
Volume is the amount of drugs which is withdrawn from the bottle to the *in vitro* tongue by µl.
Weight is the amount of drugs in the *in vitro* tongue by µg.
Sample Concentration describes the concentration of the drug when it is dissolved.

In **Table 3**, the concentrations of 1.0, 5.0 and 10 mg per ml of heroin were tested by the Marquis and cobalt thiocyanate reagents. The Marquis reagent gave positive results with concentrations of 5.0 and 10 mg/ml and

ISSN: 2789-1895 online
ISSN: 2958-3101 print

the amounts of drug on the *in vitro* tongue were 100 and 200 µg. Mandelin reagent gave positive results with methadone (LGC standard) which gave a green color. In this color test, the reagent had a dark yellow color and the amount of the drug on the *in vitro* tongue was just 20 µg which was achieved by adding 20 µL concentration of 1.0 mg per ml methadone, but because of the bright yellow color of the reagent and low rate of the drug, it was difficult to distinguish the color. A positive result was obtained with 20 µl of 1.0 mg/ml of MDMA using the Marquis reagent; a purple color was obtained from a low concentration and a small amount of MDMA. Moreover, the Marquis reagent gave a positive result with morphine in a quantity of 40 µg on the *in vitro* tongue, which gave a light purple color; however, it gave a negative result with 20 µg on the *in vitro* tongue.

Discussion

Drunk and drugged driving is a serious problem all over the world. This has significant implications for road safety because drunk or drugged driving may increase a driver's chance of being involved in a car accident as compared to a drug-free driver. Many mobile roadside drug testing devices have recently been introduced to the market as the number of drug-impaired drivers involved in crashes has increased. Oral fluid, urine or blood matrices are used in these devices [25]. Chemical spot tests have achieved great success in determining the occurrence of drugs at the roadside. Many advantages make it distinctive and able to be certified as a presumptive test, such as simple chemical reactions that lead to visible results, materials and reagents are available and inexpensive and it is easy to use by technicians without extensive training. It requires only a small number of reagents and materials and law enforcement agents can use it in the field. An oral fluid sample is the best way to apply color tests to drugs and these can be taken without embarrassment. Roadside drug testing needs to be further developed to be able to determine several different drugs in one test. In addition, low concentrations have given passive results which suggest that further research is also needed to find a method to determine the time of taking the drug, which is on-site and with different sampling processes. The main cause of the failure of the test is the reagent. It is important to test it before doing any roadside drug testing. Some reagents expire after just a few days and some have to be made daily. More, the test does not work after the passage of time after using the drug. A gradual evaluation was noted in devices that are used in the field. Some of these devices can test several drugs at the same time. On the other hand, there is not just one test which can detect all drugs at the same time and I hope this will be the focus of attention of a researcher in the future. Although other analytical techniques like FTIR are more sensitive, they require sophisticated equipment and need to be tested in the laboratory and spontaneous results could not be possible on the spot at the roadside. From the results as summarized in **Table 3** and **Figure 2**, in our opinion, the reason for the negative results was that the amphetamine was from the street and it was of unknown purity. The Marquis reagent is supposed to show an orange color for amphetamine; however, it was unable to obtain a clear result. But the Fast Blue B test showed positive results for cannabis. There was a clear difference in the ratio of the colors depending on how much of the drug concentration was on the *in vitro* tongue, but the test was very sensitive even with a small amount of the reagent. Cobalt thiocyanate reagent was successful with cocaine, which gave a clear blue color, however, it gave a very light blue color with low concentration. The amount of cocaine on the *in vitro* tongue was just 20 µg, which was supposed to be enough to apply color tests for cocaine with cobalt thiocyanate reagent, since even a very light blue color indicates the presence of the drug. The Marquis reagent was not sensitive to a low concentration and amount of codeine. However, when the amount of the drug was increased to 35 µg on the *in vitro* tongue, it gave a positive result, and a very light purple color was shown. The Marquis reagent gave positive results with different concentrations and very low amounts of the drug on the *in vitro* tongue. However, this reagent was not very sensitive with a low concentration; it gave a negative result with one mg per ml, which was the amount of 20 µL drug on the *in vitro* tongue.

ISSN: 2789-1895 online
ISSN: 2958-3101 print

The Mandelin reagent gave a positive result with methadone, but because of the bright yellow color of the reagent and the low rate of the drug, it is difficult to distinguish the result color. Further, Marquis reagent was tested with methadone, which was from a pharmacy and is used by diabetics. In our opinion, the Marquis reagent was the most widely used reagent and it gave good results with most of the drugs that were tested. However, it gave negative results to low concentrations, which indicates that it is not very sensitive with low concentrations. Cobalt thiocyanate is more sensitive to heroin in low concentrations, for which it gave clear results. Furthermore, it is observed that the reagents used for detection are specific to drugs and hence they will not interfere with the parent drug. For example, Marquis reagent was used to detect morphine, whereas for morphine derivative (cocaine), cobalt thiocyanate reagent was used. Both the reagents are specific to detect morphine and cocaine, respectively.

Conclusion: This study indicates that chemical spot tests achieve great success in determining the occurrence of drugs at the roadside. It requires only a small number of reagents and materials and law enforcement agents can use it in the field. An oral fluid sample is the best way to apply color tests to drugs and these can be taken without embarrassment. Hence the proposed techniques will help to realize the quick evaluation of the drugs and help in the regulation and prevention of accidents and antisocial activities by drug consumption.

Author contributions: MHA & PK contributed to the conceived and designed the study. MHA collected the data and SGE, RSA & SKA contributed to the analysis of data. All the authors have drafted the manuscript and revising for important intellectual context and have approved the final version.
Conflict of interest: The authors declare the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
Ethical issues: Including plagiarism, informed consent, data fabrication or falsification and double publication or submission were completely observed by the authors.
Data availability statement: The raw data that support the findings of this article are available from the corresponding author upon reasonable request.
Author declarations: The authors confirm that all relevant ethical guidelines have been followed and any necessary IRB and/or ethics committee approvals have been obtained.

References

1. Cameron M, Newstead S, Clark B, Thompson L (2022) Evaluation of an increase in roadside drug testing in victoria based on models of the crash effects of random and targeted roadside tests. Journal of Road Safety. 33 (2): 17-32. doi: 10.33492/JRS-D-20-00272
2. Pirro V, Jarmusch AK, Vincenti M, Cooks RG (2015) Direct drug analysis from oral fluid using medical swab touch spray mass spectrometry. Analytica Chimica Acta. 861: 47-54. doi: 10.1016/j.aca.2015.01.008
3. The national highway traffic safety administration standardized field sobriety testing procedures horizontal gaze nystagmus instructions. (n.d.). www.NJ.gov.
4. Verstraete AG (2005) Oral fluid testing for driving under the influence of drugs: history, recent progress and remaining challenges. Forensic Science International. 150 (2-3): 143-150. doi: 10.1016/j.forsciint.2004.11.023
5. Van der Linden G, Wille SM, Ramírez-Fernandez M, Verstraete A, Samyn N (2015) Roadside drug testing: Comparison of two legal approaches in Belgium. Forensic Science International. 249: 148-155. doi: 10.1016/ j.forsciint.2015.01.034
6. Bunn T, Singleton M, Chen I-C (2019) Use of multiple data sources to identify specific drugs and other factors associated with drug and alcohol screening of fatally injured motor vehicle drivers. Accidental; Analysis and Prevention. 122: 287-294. doi: 10.1016/j.aap.2018.10.012
7. Jenkins AJ, Goldberger BA (2002) On-site drug testing (Jenkins AJ, Goldberger BA, Eds. 2002nd ed.). Humana Press. Harvard, Vancouver. doi: 10.1385/1592592724
8. Castro A, Lendoiro E, Jiménez-Morigosa C, Cruz A, Lopez-Rivadulla M (2016) Drug detection on Spanish roadsides. Toxicologie Analytique et Clinique. 28 (2): S15. doi: 10.1016/j.toxac.2016.03.022

ISSN: 2789-1895 online
ISSN: 2958-3101 print

9. Kadehjian L (2005) Legal issues in oral fluid testing. Forensic Science International. 150 (2-3): 151-160. doi: 10.1016/j.forsciint.2004.11.024
10. Carlin M (2009) Forensic science: Roadside drug testing. Measurement and Control. 42 (10): 306-309. doi: 10.1177/002029400904201003
11. Raphael C, Wong HY (2005) Drugs of abuse; body fluid Ttesting. Humana Press Inc. ISBN: 978-1588294357.
12. Aberl F, VanDine R (2005) Saliva and sweat testing with drugwipe®. In: Drugs of abuse (pp. 161-175). Forensic Science and Medicine. Humana Press. doi: 10.1007/978-1-59259-951-6_10
13. Flanagan RJ, Taylor AA, Watson ID, Whelpton R (2008) Fundamentals of analytical toxicology: Clinical and forensic. Wiley-Blackwell. doi: 10.1002/9781119122357.ch1
14. Drummer OH (2006) Drug testing in oral fluid. The Clinical Biochemist Reviews. 27 (3): 147-159. PMC: 1579288.
15. Cole MD (2003) The analysis of controlled substances. John Wiley & Sons. ISBN: 978-0-470-86455-5.
16. O'Neal CL, Crouch DJ, Fatah AA (2000) Validation of twelve chemical spot tests for the detection of drugs of abuse. Forensic Science International. 109 (3): 189-201. doi: 10.1016/s0379-0738(99)00235-2
17. Nagy G, Szöllősi I, Szendrei K (2005) Colour tests for precursor chemicals of amphetamine-type substances. Scientific and Technical Notes. SCITEC/20. United Nations, Office on Drugs and Crime. Szeged University, Hungary.
18. Cooper G, Negrusz A (2013) Clarke's analytical forensic toxicology (2nd ed.). Pharmaceutical Press. UK. ISBN: 9780857110541.
19. Bell S (2010) Drugs, poisons and chemistry. Facts on file, Chelsea House. ISBN: 978-0816055104.
20. Moffat AC, Osselton MD, Widdop B (2011) Clarke's analysis of drugs and poisons (4th ed.) Pharmaceutical Press. London, Chicago. ISBN: 9780853697114.
21. Kapp RW (2006) Clarke's analysis of drugs and poisons, 3rd ed. (Edit. Moffat AC, Osselton MD, Widdop B) pp. 480. Publisher: Pharmaceutical Press: London. 2004. ISBN: 0-853-69473-7.
22. Samuels E (2000) Color test reagents/kits for preliminary identification of drugs of abuse. US Department of Justice, Office of Justice Programs. 1-24. NCJ Number 183258.
23. Agg KM, Craddock AF, Bos R, Francis PS, Lewis SW, Barnett NW (2006) A rapid test for heroin (3,6-diacetylmorphine) based on two chemiluminescence reactions. Journal of Forensic Sciences. 51 (5): 1080-1084. doi: 10.1111/j.1556-4029.2006.00215.x
24. Kovar K-A, Laudszun M (1989) Chemistry and reaction mechanisms of rapid tests for drugs of abuse and precursors chemicals. 2-19. UN. Division of Narcotic Drugs. Applied Scientific Research and Technical Information Section. Vienna, Austria.
25. Alhefeiti MA, Barker J, Shah I (2021) Roadside drug testing approaches. Molecules. 26 (11): 3291. doi: 10.3390/molecules26113291