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Drug driving in Italy. The results of the first roadside drug testing service utilizing on-site confirmatory analysis between 2019 and 2022

Ariana Soledad Poetto [a,*], Giulio Catesini [a,b], Paolo Dossetto [a,c], Stefano Marchetti [a], Riccardo Addobbati [a,d]

[a] Forensic Lab Service Srl, Rome, Italy
[b] Division of Metabolism, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy
[c] Sciex, Milano, Italy
[d] Institute for Maternal and Child Health-IRCCS Burlo Garofolo, 34137 Trieste, Italy

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ABSTRACT

Background: Drug driving represents a public safety concern, and the size of this issue in Italy is not fully known. Drug testing is composed of two steps: 1) screening and 2) confirmatory analysis. The second step, and the associate medical examination to assess the state of impairment, usually are not performed right after the screening as they require specialized personnel and instrumental equipment that are not historically available at roadblocks. These pitfalls make this process both complicated and time-consuming.
Methods: A mobile laboratory was set up in 2019 by the Forensic Lab Service S.r.l. (limited liability company) to improve roadblock timing, planning, as well as to shed light on the extent of the drug driving issue in Italy. Drug screenings were performed using DrugWipe® Saliva testing. Confirmatory analysis was performed on oral fluids by liquid chromatography coupled with tandem mass spectrometry. A dedicated room of the mobile laboratory was also designed for drug driving medical assessment.
Result: 2082 samples were collected during 88 road safety services held in different locations across Italy. In total, 9 % of the tested subjects were positive to both the screening and the confirmatory analysis. The most prevalent illicit drugs found in this study were THC (72 %), followed by cocaine (41 %). Drug drivers were mostly male (93 %) and younger than 30 years of age (58 %).
Conclusions: The prevalence of drivers testing positive for illicit drugs resulted to be higher compared to the results obtained in the DRUID project and to other surveys previously performed in Italy. These data demonstrate the need for control services to improve road safety in regards to drug driving.

1. Introduction

Illicit drugs may have a negative impact on driving performance. The increased risk of road accidents related to drug driving represents a public safety concern, which has been gaining more attention worldwide in the last few decades. This concern is reflected from the increasing number of studies, such as the DRUID project, publications shedding light on this issue, political agreements and the EU action plans that have been forged in recent years [1–5]. A retrospective study published in 2019 concluded that a noteworthy percentage of Italian drivers involved in road traffic crashes tests positive for alcohol (16.2 %) and illicit substances (5.8 %) [2]. From the DRUID project, drug drivers only represented 3.9 % of the study population in the Veneto region of Italy. These results included data collected during both high-risk hours, a period clearly distinguished by a known positive correlation between drug driving and accident occurrence, and low-risk hours [6]. In fact, several surveys performed in recent years reported a 10 % or higher prevalence of drug use and abuse among drivers when study surveys only included weekend nights and in precisely selected geographic locations [6]. Driving under the influence of drugs is forbidden by the Italian law and is punishable according to article 187 of the traffic code [7].

1.1. Drug testing procedures

The current drug testing protocol is a two-step procedure which

* Corresponding author.
E-mail address: ariana.poetto@hotmail.it (A.S. Poetto).

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starts with a mandatory initial drug screening. These roadside screens are occasionally performed by the police using point-of-collection immunoassay testing devices. In the event a sample tests positive for one or more drugs, the second confirmatory analysis step becomes required. The second step utilizes more accurate analytical techniques, such as gas or liquid chromatography coupled to mass spectrometry (GC-MS or LC-MS) [8] or tandem mass spectrometry (MS/MS). These are highly advanced and sensitive instruments that require specially trained personnel to carry out the analysis. Therefore, in a situation in which confirmation is required, the driver must be escorted to a healthcare facility where either a blood or urine sample is collected and analysed. The lab results are normally made available in a few days. However, the main pitfall of this confirmation sample collection procedure requiring a healthcare facility is the time that elapses between the screening and the collection for confirmatory analysis. This is highly variable and can result in a significant reduction of circulating drug levels due to drug metabolism and excretion [9]. This is particularly evident in the analysis of tetrahydrocannabinol (THC), where delayed blood tests are unlikely to confirm a drug exposure above the legally defined cut-off value [10]. The result of the confirmatory analysis, in these cases, would not be representative of the exposure of the driver to illicit substances at time of screening. This issue can be solved by collecting the sample for the confirmatory analysis on-site, right after a positive screening assessment is obtained. More recently, oral fluid sample have been validated as an appropriate bio-specimen that can be obtained on-site and then sent to an accredited laboratory and provide legally defensible result. This procedure for confirmatory analysis will have lab results available after a few days The use of oral fluid as a viable and convenient alternative specimen for drug testing [11–13] provides a rapid and non-invasive method for sample collection that can be performed on-site and does not require the intervention of a health professional. This sample collection technique is currently used for illicit drug testing in routine police practice and thus can be readily adoptable for drug driving enforcement.

1.2. A mobile laboratory for on-site drug testing and medical examination

The new guidelines drafted and approved by the Ministerial Circular of February 11, 2019, allowed to enrich the usual roadside checks with private mobile laboratories and specialized personnel for drug driving assessment [14]. The Forensic Lab Service S.r.l. (limited liability company) set up a mobile laboratory specifically for this purpose. This laboratory is arranged into three rooms, in addition to the driving cabin, as depicted in Fig. 1. Each room is equipped with independent air conditioning systems to ensure adequate temperature for laboratory function. The first room is used for medical examination and is appropriately named the “Doctor’s Room”. The second is dedicated to sample preparation, while the third room in Fig. 1, the “Instruments Room”, houses the LC-MS system that is used to perform the analyses.

Electricity to power the laboratory instruments, computers, lighting and conditioning is obtained from a portable diesel generator. This mobile laboratory was employed for both roadside screening and confirmation analyses from October 2019 until 2023. The main objectives of this publication are to introduce a novel strategy to identify subjects driving under the influence of illicit drugs, to estimate the extent of drug driving in Italy, share data regarding the frequency of drivers testing positive for one or more illicit drugs, identify trends in substance type, and finally compare these data with other studies. The prevalence rate of drugs was calculated on samples collected on road safety services held in different Italian locations as a part of routine police activities.

2. Methods and materials

In the last three years (between October 2019 and November 2022) 88 traffic stops were set up by local Police Squads in different Italian locations: such as highways, other major streets, and city roads. These checks were performed mostly during weekend nights (between the hours of 1 to 6 am), as a part of routine police road safety services. These sites for road safety services were specifically selected as they afforded drivers no possibility to avoid the road block.

2.1. Driver selection

Drivers undergoing the drug screening test were selected by the serving police officers. All motor vehicles were considered; including cars, vans, tracks, scooters, and motorcycles. All drivers were asked to voluntarily consent to undergo alcohol and drug tests. However, refusal to undergo testing is considered a formal admission of driving under the influence by Italian law.

2.2. Materials

DrugWipe® and WipeAlyser® screening devices were produced by Securetec (Neubiberg, Germany). Quantisal® or by StatSure Saliva Sampler® oral fluid collection devices were purchased from

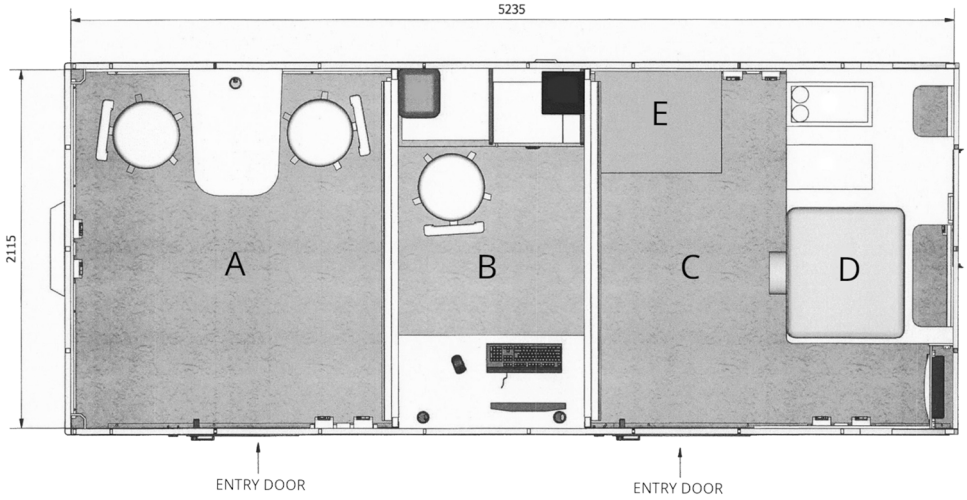

Fig. 1. Mobile laboratory arrangement, Figure 5 Mobile laboratory arrangement. Picture and project. A=Doctor’s room, B=sample preparation room, C = instruments room, D = Mass spectrometer SCIEX Triple QuadTM 5500 + QTRAP®, E = Portable diesel current generator. The distances reported are expressed in centimetres.

Immunalysis (Pomona, CA, USA) and from StatSure Diagnostic Systems (Brooklyn; NY, USA), respectively. Liquid chromatographic separations in the confirmatory analyses utilized a Hypersil Gold C18 column (1.9 μm, 125 Å,50 ×2.1 mm), provided by Thermo Fisher Scientific Inc. The mobile phase and the internal standard (IS) solution were provided by Eureka Lab Division Srl (Chiaravalle, AN, Italy). The IS used was a mixture of stable isotope standards. The full list is reported in Table 1.

2.3. Drug screening test

Drug screening was performed as required by police practice protocols using DrugWipe® Saliva testing in combination with WipeAlyser®, a hand-held electronic device employed to read DrugWipe® as to eliminate bias in the results often associated with human visual reading. The drug screen tested for a variety of illicit drugs, which included cannabis, opiates, cocaine, and amphetamines/methamphetamines. Subjects who tested positive for one or more substances in the

Table 1
Chromatographic and mass spectrometric parameters.

Analyte ID	RT (min)	Precursor ion			Product ion		
		M+H (*m/z*)	DP (V)	EP (V)	Q3 (*m/z*)	CE (V)	CXP (V)
Cocaine 1	6	304.2	120	10	182.2	27	12
Cocaine 2	6	304.2	120	10	105.2	34	12
Cocaine C13 1	6	305.2	120	10	183.2	27	12
Cocaine C13 2	6	305.2	120	10	106.2	34	12
BEG 1	4.22	290.1	95	10	168.1	25	12
BEG 2	4.22	290.1	95	10	105.0	44	12
Cocaethylene 1	6.8	318.1	100	10	196.1	28	12
Cocaethylene 2	6.8	318.1	100	10	150.0	35	12
Cocaethylene C13 1	6.8	319.1	100	10	197.1	28	12
Cocaethylene C13 2	6.8	319.1	100	10	151.0	35	12
Morphine 1	1.8	286.1	120	10	165.0	60	12
Morphine 2	1.8	286.1	120	10	181.0	48	12
Buprenorphine 1	7.8	468.2	90	10	396.1	53	10
Buprenorphine 2	7.8	468.2	90	10	414.3	48	10
6-MAM 1	3.9	328.0	140	10	165.0	53	12
6-MAM 2	3.9	328.0	140	10	211.2	38	12
Amphetamine 1	3.7	136.1	45	10	91.0	27	12
Amphetamine 2	3.7	136.1	45	10	119.0	13	12
Codeine 1	3.4	300.1	120	10	165.1	64	12
Codeine 2	3.4	300.1	120	10	153.1	62	12
MDA 1	4.1	180.2	50	10	133.1	25	12
MDA 2	4.1	180.2	50	10	135.1	27	12
MDE 1	5.1	208.2	62	10	163.1	29	12
MDE 2	5.1	208.2	62	10	135.1	25	12
MDMA 1	4.5	194.2	65	10	163.0	18	12
MDMA 2	4.5	194.2	65	10	105.0	34	12
MDMA C13 1	4.5	195.2	65	10	164.0	18	12
MDMA C13 2	4.5	195.2	65	10	106.0	34	12
Methadone 1	8.9	310.2	80	10	265.1	22	12
Methadone 2	8.9	310.2	80	10	223.1	30	12
Methadone C13 1	8.9	311.2	80	10	266.1	22	12
Methadone C13 2	8.9	311.2	80	10	224.1	30	12
EDDP 1	8	278.1	120	10	234.0	42	12
EDDP 2	8	278.1	120	10	249.1	33	12
EDDP C13 1	8	279.1	120	10	234.0	42	12
EDDP C13 2	8	279.1	120	10	250.0	33	12
Methamphetamine 1	4.2	150.1	60	10	119.1	16	12
Methamphetamine 2	4.2	150.1	60	10	91.0	29	12
MBDB 1	5.4	208.2	60	10	135.2	31	12
MBDB 2	5.4	208.2	60	10	177.0	15	12
THC 1	8.6	315.2	50	10	193.2	33	12
THC 2	8.6	315.2	50	10	123.2	45	12
IS Codeine-D6	3.5	306	120	10	165.2	60	12
IS Cocaetilene-D3	6.8	322.2	120	10	200	28	12
IS Amphetamine-D11	3.7	148	55	10	99	29	12
IS MDA-D5	4.1	185	65	10	138	26	12
IS Methamphetamine D11	4.2	162.1	90	10	128.2	16	12
IS MDMA-D5	4.5	200	80	10	166	20	12
IS MDE-D6	5.1	214	90	10	164	20	12
IS Buprenorphine-D4	7.8	472.2	80	10	400.2	55	12
IS THC-D3	8.6	318.2	80	10	196.2	32	12
IS 6-MAM-D6	3.9	334.2	120	10	211	40	12
IS COCAINA-D3	6	308.1	90	10	186	28	12
IS MORFINA-D6	1.8	292	120	10	181	50	12
IS METADONE-D9	8.9	320.3	90	10	269	22	12
IS BEG-D8	4.22	298	114	10	171	27	12
IS EDDP-D3	8	282.2	120	10	235	42	12

Table Cromatographic retention times (RT) and compound dependent parameters: precursor ion mass (Q1), product ion mass (Q3), declustering potential (DP), entrance potential (EP), collision energy (CE), and cell exit potential (CXP). BEG = Benzoylecgonine; 6-MAM = 6-Monoacetylmorphine; MDA = 3,4-Methylenedioxyamphetamine; MDE = N-methyl diethanolamine; MDMA = 3,4-Methylenedioxymethamphetamine; EDDP = 2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; MBDB=N-Methyl-1-(1,3-Benzodioxol-5-yl)−2-Butanamine; THC=Tetrahydrocannabinol.

DrugWipe® Saliva testing were subsequently asked to provide two additional oral fluid samples. Each 1 ml sample was collected employing either a Quantisal® or StatSure Saliva Sampler® collection device. One of the two samples was used for on-site confirmation analysis immediately after collection, while the second was sent by the chain of custody to the Pharmacotoxicology Unit of the health district Azienda Sanitaria Locale of Pescara as required by law. This second sample is stored for a period of six months and is only tested in the event further analyses are required. Both oral and written information was provided to the drivers prior to the screening test as informed consent is legally required before the confirmatory analysis and the medical assessment can begin.

2.4. Confirmatory analysis

Confirmatory analysis was performed by a forensic analyst directly on-site in the mobile laboratory which was equipped with a SCIEX Triple QuadTM 5500 + QTRAP® Ready mass spectrometer coupled with UHPLC module ExionLC® provided by Sciex, a part of Danaher (Washington, D.C., USA). The LC-MS/MS (liquid chromatography coupled to tandem mass spectrometry) method employed was developed and validated by Bassotti et al. [15]. This method was very efficient for on-site application as it had 12-minute sample run times. This method allows the confirmation of the exposure to all the drugs screened by DrugWipe® Saliva testing in a short turnaround time (approximately 30 min), as multi-standard calibrators were simultaneously used in every run [15]. Briefly, each 1 ml oral fluid samples collected on-site was directly diluted with the preservative buffer contained within each of the collection device. A methanolic solution containing the analytes isotopologues employed as an IS was then used to induce protein precipitation. A four-fold dilution factor resulted from both steps. As previously stated, the specific components of the IS are listed in Table 1. The samples were vortexed after dilution and then centrifuged at 14, 000 rpm for 10 min. A 200 μL aliquot of supernatant was transferred to an autosampler vial and kept at 15 °C until analysis. Reverse phase liquid chromatographic separation was performed at 40 °C through mobile phase B (MeOH added with 0.1 % formic acid) gradient that ranged from 5 to 100 %. Mobile phase A was comprised of ultrapure water with 0.1 % formic acid. The gradient was followed by washing and re-equilibration steps so the column can be used for multiple same runs. The separated analytes were positively ionized in the electrospray ionization (ESI) source and then fragmented to obtain two selected transitions for each analyte. The multiple reaction monitoring (MRM) transitions (precursor and product ions) used for the detection of each analyte are reported in Table 1. Analyte retention times and compound-dependent parameters are also provided. A single transition was selected for the stable isotopes of each analyte present in the IS. The resultant data were processed by version 1.6.3 of Analyst software, while analytes quantification was performed by Sciex OS (software package AB SCIEX). The cut-off levels used to define the result as positive for recent drug use were defined by several internationally recognized authorities where available. These international authorities included the National Institute of Health (NIH), the Substance Abuse and Mental Health Services Administration (SAMHSA), the European Workplace Drug Testing Society (EWDTS), and the Italian Group of Forensic Toxicologists (GTFI). These cut-off values are listed in Table 2, but were updated as the corresponding guidelines were modified over the course of the study duration [11–13,16]. However, GTFI cut-off values are considered to be the general evaluation standards for confirmation test results according to Italian law. For cocaine, positivity was assessed if both cocaine and BEG (its main metabolite) were found in the sample at concentrations above the cut-off. The analytical range of the LC-MS/MS method employed included the reported SAHMSA, EWDTS, and GTFI cut-off levels for all the analytes, except for EDDP (a major metabolite of methadone), where NIH guidelines were applied (current version). It must be noted that analytical sensitivity of the tests applied in this study was not currently of concern as most of the samples analysed revealed significantly high concentrations of the analytes of interest. Conversely, the problem was quite the opposite, as some samples had concentration levels that were far higher than expected and resulted in detector saturation. This phenomenon is easily recognisable as characteristic peak shape distortions occur (Fig. 2). To overcome this saturation issue, the applied protocol used the M+ 1 isotope as a surrogate for quantification instead of the most abundant isotope (M). As reported by Wei et al., the signal intensity of this M+ 1 species is approximately five times lower than M, and thus usually not involved in detector saturation issues [17]. This allows for analyte quantitation without the need to perform a sample dilution and sample re-run. The MRM transitions used for M+ 1 isotopes, together with the compound-dependent parameters employed, are also reported in Table 1.

Table 2
Cut-off levels employed for confirmatory analysis.

Analyte	Cut off NIH* (ng/ml)	Cut off NIH** (ng/ml)	Cut off SAHMSA, EWDTS and GTFI (ng/ml)
Amphetamine	50	30	15
Metamphetamine	50	30	15
MDA	50	30	15
MDMA	50	30	15
MDEA	50	30	15
THC	2	2	2
Cocaine	8	8	8
BEG	8	8	8
Cocaethylene	-	-	-
Morphine	40	40	15
Codeine	40	40	15
6-MAM	4	4	2
Methadone	-	20	20
EDDP	-	20	-
Buprenorphine	-	5	1

Cut-off levels employed for confirmatory analysis established byItalianNIH: Cut off NIH* = Cut off reported by NIH based on SAHMSA and GTFI (previous versions); Cut off NIH** = Cut off NIH based on EWDTS (previous version), Cut off SAHMSA, EWDTS and GTFI=Cut off values published by the Substance Abuse and Mental Health Services Administration (SAMHSA) in 2019, the European Workplace Drug Testing Society (EWDTS) in 2022, and the Italian Group of Forensic Toxicologists (GTFI) in 2022.

2.5. Medical examination

Medical examination was performed for all drivers testing positive in the initial screen. Among others; heart rate, blood pressure, state of consciousness, presence of memory deficits, delirium and hallucinations, behaviour, language, balance and posture were all evaluated to assess impairment in drug driving cases.

2.6. Data collection and analysis

All the records were anonymized prior to analysis. The collected testing results, which consisted of both the initial screening and LC-MS/MS confirmation, were stored in an excel database and presented as positive or negative. Additional basic information recorded included the date and place of sample collection. Demographic data of the drivers who tested positive were also collected to investigate age and gender trends in relation to driving and illicit drug consumption. Descriptive statistics were used to present and analyse the data recorded. The number of positive confirmation tests was compared with the total number of samples collected to estimate the frequency of drivers who tested positive for one or more illicit drugs. The proportion of subjects who tested positive to each analysed substance was also determined. In this study, false positive results were defined as positive screening tests not confirmed by LC-MS/MS analysis. The percentage ratio between the number of negative events wrongly categorized as positive (false positive results) and the total number of actual negative tests was also

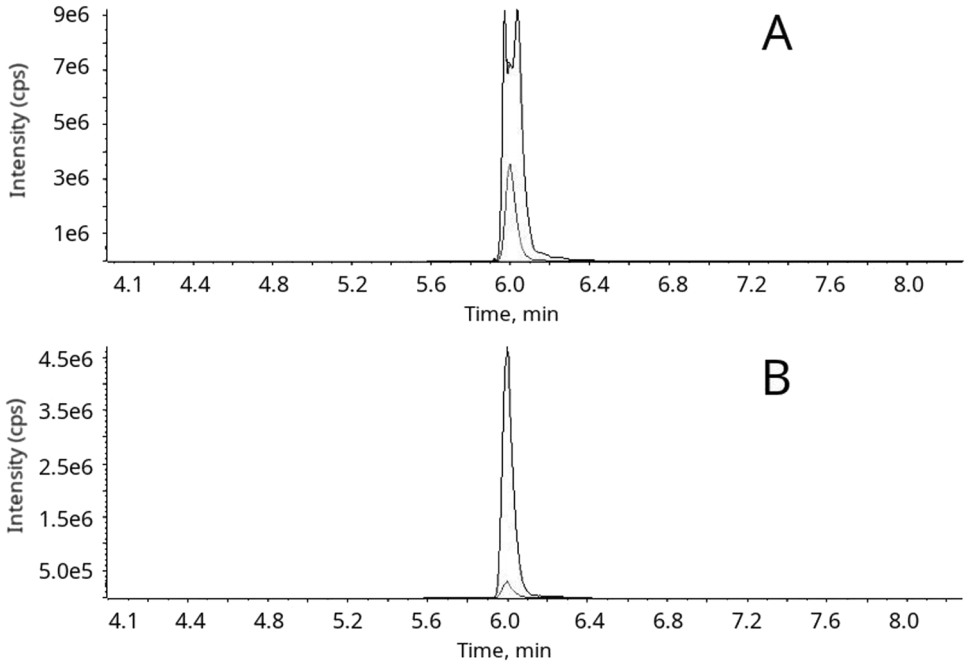

Fig. 2. Peak distortion due to detector saturation and M+1 regular shape. Use of a less abundant isotope (M+1) to overcome detector saturation. A=Peak shape distortion caused by detector saturation by the most abundant isotope, B = M+1 regular peak shape.

calculated to estimate the false positive rate.

3. Results

Over the period investigated, 2082 drivers were screened for illicit drugs. Among all the drivers, 188 (9 % of the subjects) tested positive to both screening and on-site confirmatory analysis. All other tested subjects were considered negative. False positive results were defined as positive screening tests that were not verified by LC-MS/MS analysis (i.e. LC-MS/MS results were equal to zero or under the cut-off level). Among all positive samples, the most prevalent illicit drug found was THC (73 %), followed by cocaine (40 %). Both THC and cocaine were found in 24 samples, shown in Fig. 3. This association represents the vast majority of polysubstance use cases (13 % of all the positive samples). This study only has one case of THC associated to opiates and one case of cocaine detected in the presence of MDMA (3,4-Methyl enedioxy methamphetamine). These were also only two samples that tested positive for opiates and MDMA, respectively. These findings are also shown in Fig. 3.

Concerning age and gender, 93 % of the drivers testing positive for illicit drugs were male. The birth-date of each drug driver was registered at the time of sample collection except for two persons whose ages were unknown. The majority of these individuals were in the youngest age group defined in this study, 18–30 years (58 %). As shown in Fig. 4, the number of drug driving subjects in each of the upper age groups was significantly lower: as 19 % were 31–40, 14 % were 41–50, and just 7 % were 51–70 years of age. No difference could be deduced between any age groups in the consumption patterns regarding the two-main illicit substances detected, cocaine and THC. Samples testing positive for cocaine demonstrated concentrations within the range of 15 – 37000 ng/ml, with a median value of 1018 ng/ml and a mean value of 3629 ng/ml (SD= 7073). THC positive samples had concentration levels between 5.8 and 3421 ng/ml. These samples had a median value of 95.00 ng/ml, while the mean concentration of THC positive samples was 312.5 ng/ml (SD=617). The false positive rate, defined as the ratio between the total number of false positive results and the total number of actual negative tests, was calculated and found to only be 3 %. Regarding amphetamines, except for the previously reported single case where of MDMA being detected in the presence of cocaine, the remaining positive screening results (17 samples) were not confirmed by LC-MS/MS analysis and were therefore defined as false positive. In other words, other than the above single case, drivers presenting as positive to MDMA at the initial screening test were then change to negative as the concentration calculated by the confirmation test was either equal to

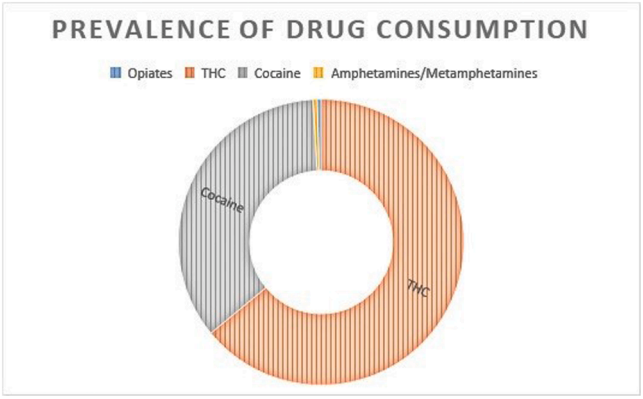

Fig. 3. Prevalence of the substances. Prevalence of the substances found in positive samples.

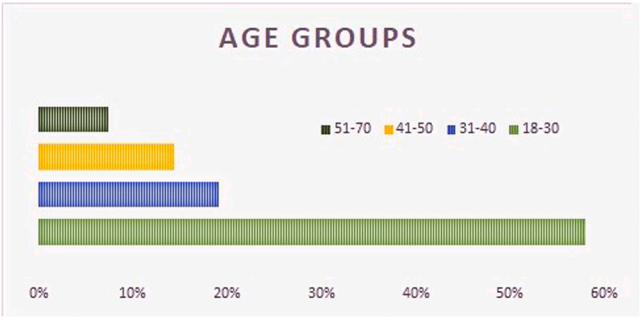

Fig. 4. Positive subjects by age group. Prevalence of drug use among the different age groups.

zero or lower than the legally established cut-off. The true positive rate is reported for all the substances in Table 3. Driving impairment was confirmed for all the drivers who tested positive for other illicit drugs.

4. Discussion

Among the 2082 samples collected and analysed by the mobile laboratory, the prevalence of positive results was 9 %. These data were compared with those obtained in the DRUID project, which is a study carried out over 5 years in 18 different European countries which aimed to evaluate the actual impact of psychoactive drugs on road safety. The prevalence of illicit drug use while driving reported in our study is similar to the data reported from Spain (8.2 %), which was the highest rate reported in the DRUID project, but was more than twice the percentage reported in the Italian DRUID report (3.9 %) [4,6]. It would be tempting to explain this discrepancy by analysing the different methods used by each country participating in the DRUID project. Specifically, the overall number of Italian samples collected in the DRUID project was much lower (n = 1310) and road blocks were performed during weekdays, weekends and included both high- and low-risk hours. However, 65 % of the samples were collected during high-risk hours (22:00–03:39 am) [6]. Instead, as explained in Section 2 above, the mobile laboratory services were performed mostly by night (1–6 am) during weekends. The different service hours could partially explain these divergent percentages as a higher number of samples were collected during the night, a period of time in which the prevalence of drug use is more commonly reported, not only in Europe but also in the USA in 2013–2014 [18]. In fact, the National Roadside Study of Alcohol and Drug Use by Drivers found that the percentage of positive tests for illicit drugs was 9.3 % during day hours and 13.2 % during night hours [18]. However, a comparison between our data and the results of this study cannot be properly done as the design of this U.S. study radically differs from its European counterpart. Particularly, in the National Roadside Study of Alcohol and Drug Use by Drivers as study participation was completely voluntary [18]. In our survey an individual's participation was voluntary, but refusing to undergo on-site alcohol and drug testing translated into a formal admission of driving under the influence, and punishable according to article 187 of the Italian traffic code [7]. It can therefore be said that participation was somewhat mandated. Interestingly, older Italian drug driving surveys show a quite higher drug driving prevalence. Particularly, it was found in 2004 that the prevalence of drug drivers was higher than 12 % as reported by the Italian police. This data is more aligned with our results, but this is just an estimate as the number of drivers stopped in 2004 was not registered [6]. Concerning the participants demographics, the vast majority of the drug drivers tested by the mobile laboratory were male, which was in line with the findings of the DRUID project [3,6]. Among all the road safety services performed by the mobile laboratory during the study period, a clear age distribution had been observed as more than half of the drug drivers were in the 18–30 age group (58 %). Even among the Italian participants in the DRUID project, the highest prevalence for drug use while driving was found in this younger age group. In that project, the youngest age group was 35–49 [6]. The Italian report of the DRUID project describes the possibility of a selection issue to explain the paucity of participants aged 50 or more due to the selection performed by the police and enforcement focusing more on higher risk subjects [6]. This schema was also used in the mobile laboratory services so that, in both cases, the participants are not representative of the general driving population. Among the positive results, the most frequently detected drug in the mobile laboratory was THC (73 %), followed by cocaine (40 %) [6]. This was exactly the opposite of DRUID project findings, in which cocaine was found to be more represented than THC. In addition, based on our data, the percentage of drivers tested positive for opiates and for MDMA was extremely low (only 1 out of 213 total positive results for all the substances). In the Italian report of the DRUID project the prevalence of drivers tested positive to illicit opiates was higher (approximately 4 out of every 52 drug drivers) [6] and amphetamines were not found among any of the samples collected. We wonder if this difference could derive from regional trends, as the Italian report of the DRUID refers to road blocks performed in the Veneto region, although the authors assess that the results may be representative for Italy as a whole [6]. The detection of two or more drugs in the same sample resulted to be significant (14 %) in our study and was in all cases involved the combination of cocaine with THC, except for one case of MDMA being found in association with cocaine, and in another single case where opiates were detected together with THC. In the DRUID project, drug-drug combinations were very rare [6]. A significant number of false positive results for amphetamine was obtained with the DrugWipe® screening test, whereas the single positive screening test result for MDMA was confirmed with LC-MS/MS. This large number of false positive results from the screening tests, which consists of an immunological assay, is possibly due to unknown cross-reactions with substances other than the amphetamines tested for during confirmation. The false positive rate cannot be justified by the cut-off levels used by the mobile laboratory, as these values were reported by SAHMSA, EWDTS and GTFI guidelines and were lower compared to those recommended by the DRUID project [4]. Regarding the practical aspects of the presented service, turnaround times were not provided for the DRUID project, but we can estimate it was in the range of 1 to 3 days as all the participating surveys had no analytical equipment nor personnel services available on-site at the road blocks. This study employed mobile laboratory services, providing confirmatory testing of oral fluid samples that were analyzed immediately after collection. The LC-MS/MS analytical method employed had run times that were able to provide comprehensive results from a sample in 30 min.

Table 3
Prevalence of the substances for which at least one sample resulted to be positive.

Name of the drug	Number of		True positive %	False positive %
	Positive screening tests	Confirmed by LC-MS/MS analysis		
THC	154	137	89	11
Cocaine	98	75	77	23
MDMA	18	1	6	94
Opiates	3	1	33	67

Table 2. Prevalence of the substances for which at least one sample resulted to be positive, true positive and false positive rate.

4.1. Limitations

Regarding the limitations of the presented study, driver selection had been carried out mostly on weekends and exclusively at night. Such parameters make unfeasible the identification of differences in drug use between day and night time frames, or between weekdays and weekends, unfeasible. Furthermore, due to the large number of locations where the roadblocks where held, it is impossible to deduce annual trends, geographical and gender variations. As in the DRUID project, the selection of drivers was probably not representative for random road traffic as drivers were selected at the discretion of the police officers serving at each roadblock. A non-negligible percentage of false positive results was observed in this study, especially for amphetamines (Table 3). These false positives are unlikely due to a higher perceived sensitivity of the screening test compared to the confirmatory LC-MS/MS analysis. The most reasonable root cause of this phenomenon would be cross-reactions in the immunoassays used in the initial screen, affecting the accuracy of those results. These molecular interactions are not reported by the manufacturer and should be investigated further to refine the test and improve the protocol and reduce the prevalence of false positive results as this will reduce cost and waste less resources. In recent years a considerable number of new psychoactive substances has

been identified within the European drug trafficking (e.g. synthetic cannabinoids) [19]. These drugs are not yet screened during road safety controls as this requires an adaptation of the national legislation and the development of dedicated analytical methods. As a consequence, the reported proportion of subjects driving under the influence of illicit drugs in this study is probably widely underestimated.

5. Conclusions

To the best of our knowledge, the service presented here is the first to include both the equipment for confirmatory analysis and medical examination at road blocks for drug driving detection. The use of an on-site mobile laboratory allows for a complete and unequivocal identification of impaired drivers in a short time frame. This also circumvents issues and variation that may result from the time delays associated with suspect transport to a medical facility. The LC-MS/MS method employed in this study for confirmatory analyses were determined to be both reliable and sensitive. The applied method even mitigated peak distortion due to detector saturation, issues associated with extremely high concentration samples. By using the M+ 1 isotopes, quantitation could be achieved from these samples without the need for sample dilution or re-run. The significant proportion of positive samples confirms that there is a concerning number of people in Italy that drive under the influence of one or more illicit drugs. These findings clearly support the need for on-site control services, as presented here, for the purpose of improving road safety.

CRediT authorship contribution statement

Paolo Dossetto: Supervision, Writing – original draft. **Stefano Marchetti:** Resources, Supervision. **Ariana Soledad Poetto:** Conceptualization, Data curation, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Giulio Catesini:** Conceptualization, Data curation, Investigation, Writing – original draft, Writing – review & editing. **Riccardo Addobbati:** Data curation, Investigation, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

All authors have no conflicts of interest and no financial interest/arrangement with one or more organizations that could be perceived as a real or apparent conflict of interest in the context of the subject of this article.

They agree to inform Forensic Science International of any conflict of interest that might arise, particularly any financial agreements they may have with pharmaceutical or biomedical firms whose products are pertinent to the subject matter dealt with in the manuscript.

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