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Original Research

Job and social problems due to morphine positivity: abuse or food contamination?

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Abstract:

Objective: After alcohol, opium is most likely the second substance that humans have been using for the longest time for its psychoactive effects: for example, morphine has the ability to decrease pain sensitivity even at small doses. The prolonged use of these substances with analgesic-narcotic action (of natural and synthetic origin), both for medical and pleasure use, causes addiction/dependence and thus is regulated by specific laws.

In Italy, in order to maximize toxicological controls, employees of public administration or private companies are subjected to constant toxicological analyses. When a worker tests positive for a certain illicit substance, they are temporarily relieved of the assignment, destined for therapeutic paths at drug addiction medical services and, in extreme, fired.

The present work wants to focus the attention on an increasingly growing and widespread problem, concerning the positivity of subjects for opiate substances, especially morphine, not as the result of illicit use/abuse but due to an involuntary intake via food, such as ingesting poppy seeds.

For this purpose, the study first describes a real case of a worker positivity for morphine related to ingestion of bread with poppy seeds that, subsequently, gave the basis for a university research program.

Materials and Methods: During the research breads with poppy seeds were consumed by twenty-two healthy subjects of both sexes (eight women and fourteen men), having different physical characteristics and lifestyles and aged between 25-73 years old. Their urine samples were tested with the classical GC/MS methods searching for opiates. Poppy seeds, manually shredded, were also subjected to toxicological analysis to verify the presence or the absence of opiates.

Results and Conclusion: The results obtained confirmed that the positivity of the subjects to morphine was not due to an abuse of the substance itself (or heroin), but from food contamination.

Keywords: Bread with Poppy seeds; Morphine; GC-MS; Food contamination; Job placement and social life.

HIGHLIGHTS

- Employees positive for morphine following toxicological analyses on workplace;
- Social and working problems for workers who tested positive

during forensic toxicological analyses;

- Commercial bread with poppy seed consumption may cause the presence of morphine in urines;
- Positivity for drugs can be also due to accidental food contamination.

Introduction: Opiates and their metabolism *Papaver somniferum L.* is an angiosperm dicot in the family *Papaveraceae*. It is an annual plant, mainly cultivated in the northern hemisphere. Its glaucous leaves have an elliptical shape, with lobed or dentate margins, & present an alternate arrangement [1].

According to manuscripts dated to about 5000 BC, opium was used and cultivated by Sumerians, the inhabitants of the ancient Mesopotamian region that corresponds to current Iraq and Kuwait [2]. Later, ancient Greeks called it *"õplo√"* (opium) and the name remained unchanged over time [3–7].

There is a lot of literature about opium poppy cultivation, chemical features, medical usage and the wars for its trade [2,8–17]. The word "*opium*" comes from the ancient Greek and means "*juice*" [1]. Indeed, it is the lactescent exudate of the immature seed capsule of *Papaver somniferum* and it can be collected by cutting the plant.

Dried opium contains about 25% of alkaloids and 10% of morphine [3,14].

After alcohol, opium is most likely the second substance that humans have been using for the longest time for its psychoactive effects: for example, morphine has the ability to decrease pain sensitivity even at small doses [1,18]. The prolonged use of these substances with analgesic-narcotic action (of natural and synthetic origin), both for medical and pleasure use, causes addiction.

In the last two centuries opium abuse, via oral assumption or smoke, was very common. However, after the discovery of the active principle of morphine (in 1803) and the subsequent legal restrictions [3], opium assumption decreased drastically. As a counter effect, the abuse of synthetic morphine-like substances took hold in occidental industrialized countries.

Morphine is widely considered the prototype of drugs with narcotic-analgesic action and it is the standard used for comparison with other painkillers [19].

Like other alkaloids, morphine has a high affinity for δ, κ and μ opioid receptors [20]. Most of the analgesic effect is due to its

binding to the latter in central nervous system (CNS) and peripheral nervous system (PNS) [21]. As a result, the descending inhibitory pathways of CNS activate and signal transmission in afferent nociceptive neurons of PNS is inhibited. This leads to the decrease of overall nociceptive signal transmission that manifests through analgesia, sleepiness, mood changes and mental confusion. Moreover, morphine is a powerful respiratory depressant [3]. The molecule is poorly soluble in water, while it has a high affinity for both acid and basic solutions (respectively as a protonate base and as hydroxyl group salt in C3 position). It is usually conserved and utilized in salt form [22,23]. In medical practice, morphine administration can occur orally (PO) or through injection, specifically: intravenous (IV), intramuscular, epidural, or intrathecal injection. Morphine suppositories are also available [24,25]. Morphine maximum concentration in plasma following oral absorption is 5-10 times lower than the one resulting from parenteral administration and it is measurable between 30 and 90 minutes after assumption [22,23,26–29]. This is because first pass effect is strong in case of oral administration and thus the average bioavailability is 30-40%, even though it can vary a lot (19-47%) [22,26–28].

Despite morphine being poorly lipophilic, it widely distributes in the whole body and its distribution volume varies between 2.1 and 4.0 L/kg [26–28,30,31]. In particular it tends to accumulate in parenchymatous tissues such as lungs, spleen and liver [3,22,26,30,32]. A part of the whole amount of circulating morphine binds to specific plasmatic proteins like albumin and γ-globulins [3,33].

Concerning metabolism, morphine is predominantly glucuronidated in the liver. This phase II reaction increases morphine solubility in water to facilitate its excretion. In particular, about 90% of morphine is converted in metabolites: morphine-3-glucorinide (M3G) (45-55%), the main product, morphine-6-glucorinide (M6G) (10-15%) and other substances in lower percentages [34]. The first pass effect following oral administration of morphine leads to a higher amount of glucuronide metabolites production with respect to other routes of administration and after 75 minutes M3G concentration is maximum [26]. One hour after oral administration, M3G and morphine

ratio is about 25:1 [28]. Morphine-6-glucuronide (M6G) is an active metabolite of morphine and, after a single dose, is detected in plasma in lower concentrations than M3G [26]. M6G binds to opioid μ receptors and it has a stronger analgesic action compared to morphine [35–37]. Moreover, it seems M6G causes less side effects [38,39]. M6G reaches its peak in concentration 45 minutes after intravenous injection and it corresponds to 25% of initial morphine concentration [26]. Instead, in case of oral administration of morphine, M6G maximum concentration is four times higher than the initial drug concentration and their ratio is 2.5-10:1 after multiple doses [3,26].

Furthermore, M6G likely accumulates in central nervous system and is responsible for the increase in power and efficacy of morphine in case of multiple administrations. The half-lives of M3G and M6G are 2-4 hours after intravenous injection and 9-10 hours following oral administration. Both metabolites are eliminated by renal excretion and their half-lives increase in case of renal insufficiency, thus leading to M3G and M6G accumulation following multiple administrations of morphine [40,41].

Heroin is an opioid drug synthesized by A.C. Wright in 1874 by acetylation of morphine [42,43]. After assumption, heroin is rapidly hydrolysed to 6-monoacetylmorphine (6-MAM) and the latter is hydrolysed to morphine. The bond of ester groups causes a structural change that increases lipophilicity of both heroin and 6-MAM [44,45]. As a result, heroin and its metabolite penetrate faster in brain tissue [33,46–48] and this contributes to a stronger pharmacodynamic effect with a much more rapid onset compared to morphine. Nevertheless, since opioid receptors are stereo-specific, heroin has a lower affinity for them with respect to its metabolites that do not have any conjugates to hydroxyl group in C3 position (like 6-monoacetylmorphine, morphine and morphine-6-glucuronide) [49,50]. Therefore heroin is usually considered as a pro-drug whose action is mainly due to its metabolites [49,51]. After administration through various routes, circulating heroin is hydrolysed to 6-monoacetylmorphine by blood and tissue esterase, in which it accumulates [3,33]. In liver, 6-MAM is hydrolysed to morphine and then the enzyme 5’-diphosphateglucuroniltrasferase (UGT) catalyses the binding of

glucuronide conjugates in position C3 or C6. The half-lives of heroin glucuronide metabolites are 2-6 hours and their long permanence in blood is due to enterohepatic recirculation. Specifically, both morphine and its metabolites follow this pathway: after biliary elimination of glucuronide metabolites, the latter are again hydrolysed to morphine by β-glucuronidases of colon microbiota [52]. Thus, new morphine enters the blood circulatory system [52]. Eventually, 5-8 hours after intravenous injection, 70% of heroin is eliminated with urines in 6-MAM, morphine and glucuronide metabolites form [53,54]. Also other heroin by-products, such as normorphine-glucuronide, codeine, morphine-3,6-diglucuronide and morphine-3-ethereal sulfate can be present in minor quantities [3,33,55,56].

1.1 Italian legislation about worker's toxicological controls

The current Italian legislation provides that some categories of workers involved in tasks with security, health, and safety risks of third parties must undergo:

- Analyses to verify the absence of occasional or habitual assumption of narcotic substances (i.e. opiates, cocaine, cannabis, amphetamines, hallucinogens) and psychotropic substances (i.e. benzodiazepines, barbiturates);
- Controls to ensure the respect of the ban on workplace drinking and to assess the absence of alcohol use disorders.

These verifications are an obligation of the employer and are carried out by the assigned competent doctor as a part of sanitary surveillance for health and safety protection of workers (art. 125 c. 1 D.P.R. n. 309/1990 e s.m.i.; Intesa Stato-Regioni 30/10/2007; Accordo Stato-Regioni 18/9/2008; art. 41 c. 4 D.Lgs. n. 81/2008 e s.m.i.) [57–60]. The lists of restricted substances and of risky jobs are definite (all. 1 Intesa 30/10/2007) [58] and the sanitary surveillance program (art. 41 c. 2 D.Lgs. n. 81/2008 e s.m.i) [60] includes a periodic medical check-up (usually once a year) upon request of the worker and in case of job change. As regards the assumption of narcotic and psychotropic substances, the medical assessment includes a physical examination and toxicological analyses on urine sample. In the event that in-depth diagnostic analyses are required (for example in case of positive toxicology test), the competent doctor directs the worker to the local chemical dependency service, called Servizio per le Dipendenze

(Ser.D.). Ser.D. is also the reference structure for the management of potential therapy/rehabilitation program of the subject.

1.2 Case report

In 2015 a railway employee was called to provide a urine sample for the routine forensic toxicological test to detect the possible presence of illicit substances. The urine sample was split into three aliquots, named "A", "B" and "C". Aliquot "A" was used to perform a preliminary immunoenzymatic assay, which turned positive for opiates (with a value equal or higher than the 300 ng/ml cut-off value). Hence, aliquot "B" underwent a confirmatory test since the preliminary screening assay can give false positives. A gas chromatography/mass spectrometry (GC/MS) analysis was performed in order to detect single molecules with high specificity and ionic-level resolution. In this case only morphine was detected with a concentration higher than the 100 ng/ml cut-off value. Eventually aliquot "C" was used to perform a GC/MS counter-analysis to further investigate the positive result upon request of the patient. The results indicated the presence of morphine and codeine, respectively with a concentration of 282 ng/ml and 119 ng/ml (cut-off value: 100 ng/ml), while no 6-MAM metabolite was detected. In addition, hair test was performed by splitting the keratin matrix into three proximal and three distal centimetres and it turned negative for opiates. The subject declared that he had never taken illicit substances or opioid medicines, but he had ingested some bread with poppy seeds the night before the drug screening. Anyway, job suspension without pay occurred according to the law and lasted for several months, until it was empirically demonstrated that the positivity for morphine was due to the ingestion of the bread itself. Clearly, that bread contained poppy seeds that had not been treated according to the European food hygiene and safety regulations.

1.3 Research project on morphine positivity in commercial bread with poppy seeds

In 2020 breads with poppy seeds were consumed by twenty-two healthy subjects of both sexes (eight women and fourteen men), having different physical characteristics and lifestyles, and aged between 25-73 years old. Their urine samples were tested with the classical analytical chemistry methods searching for opiates.

All the participants (healthy, non-drug users or under pharmacological therapy) were volunteers and signed an informed consent. All of them strictly followed the rules of the experimental design, developed according to the scientific method.

Refer Figure 1 here - Bread with poppy seeds bought at the supermarket.

MATERIALS AND METHODS

2.1 Samples collection

Each volunteer was provided with two sterile urine containers and a loaf of bread with poppy seeds. The latter was ingested after collecting a urine sample, which was used as a blank for further comparisons. All the participants consumed the bread the night before the forensic toxicological screening. The respective urine samples, fourty-four in total, were collected the next day.

2.2 Urine samples extraction

An aliquot of 4 ml of urine sample was added with internal standards, specifically scopolamine and nalorphine at 1000 ng/ml, and 4 ml of HCl 0.1 N for hydrolysis. Then samples were put in a centrifuge and the supernatant was collected for extraction. Solid-phase extraction was performed with the SPEC-3mL-MP1 system, which consists in a column that contains fiberglass and silica packed discs. MP1 (Mixed Phase 1) acronym indicates the simultaneous presence of non-polar groups and cationic exchange groups. SPEC column was inserted in a vacuum manifold for activation: vacuum was between 5 and 10 mmHg, for the good interaction between the solution and the solid phase of the column. Moreover 1 ml of methanol and then 1 ml of HCl 0.1 N were added to activate the SCX (Sulfonic) groups present in the column. After activation, also the hydrolysed sample was injected into the column and several steps of aspiration and washing were performed by adding, respectively, 1 ml of HCl 0.1 N and then 1 ml of methanol. Eventually, 1.5 ml of elution solvent, which contained dichloromethane, isopropanol, and ammonia, was added to the column. The eluate was collected into a vial and the organic solvent was evaporated under nitrogen flow. After that, 50 µl of MSTFA were added for derivatization and the vial was placed in a thermostatic stove at 70°C before resuspension with 250 µl of dichloromethane.

2.3 Poppy seeds extraction

To corroborate the hypothesis that the presence of morphine traces in urine samples was due to the consumption of bread with poppy seeds, a qualitative analysis was

performed on poppy seeds themselves. After accurate mechanical extraction, the seeds were weighted (3.908 grams) and shredded in a mortar. Extraction solvent (26 ml of ethanol) was added in excess to the seed powder, which was subsequently put in ultrasonic bath for 30 minutes. Then the homogenate was filtered through filter paper and left to evaporate. The resulting product was an oil with a "dirty" matrix that contained various molecules of plant origin. To extrapolate only morphine, a selective extraction was performed by adding 3 ml of HCl 0.1 M and 1000 ng of both internal standards to the oily mixture. After that, the sample was centrifuged (2000 g for 10 minutes) and the aqueous phase was recovered. The latter underwent a solid-liquid extraction with SPEC-MP1 columns, as described in the previous paragraph for urine samples extraction.

2.4 GC/MS analysis

The analysis was performed with the Agilent 5975C Inert MSD-MS 7890 A gas chromatography machine and single quadrupole mass spectrophotometer system, equipped with the CTC PALL SAMPLER 80 automated sampler. Concerning the column, the HP-5MS UI 30 m x 0,250 mm model, with a run-time of about 30 minutes, was used. Mass spectra were acquired in Selected Ion Monitoring (SIM) mode.

Refer Table 1 here– Retention Time (RT) and Ion rate (m/z) of the considered molecules.

2.5 LOD and LOQ

First, a calibration curve was built with the internal standards, to quantify the concentration of morphine in each sample. Moreover, a mixture of all the molecules under study, at proper concentration, was added to blank samples to evaluate the analytical sensitivity of the technique to each drug and its metabolites. After that, limit of detection (LOD, that is 5 ng/ml) and limit of quantitation (LOQ, that is 10 ng/ml) were determined. Limit of detection is the minimum concentration necessary to perform a qualitative analysis to detect a specific analyte. Instead, limit of quantitation is the maximum concentration up to which it is possible to obtain a quantitative measurement with the respective uncertainty and is higher than LOD. The range between the two limits (LOD and LOQ) in a calibration curve can discriminate only the presence or the absence of the analyte.

These samples were used to build a six-point calibration curve in the range of 10-500 ng/ml (10, 20, 50, 150, 300, 500 ng/ml). In

particular, considering the cut-off values suggested for morphine, the curve was constructed starting from 10 ng/ml value. Linear correlation coefficients for the molecules under study were optimal ($R^2 \geq 0.9977$).

Refer Figure 2 here – The calibration curve and R^2 value.

After the chemical analysis of the substances under study and the construction of the calibration curve from the obtained data, it was assessed that the concentrations were lower enough than the standard cut-off values for urine matrix generally used by the laboratory. Specifically, the cut-off value for morphine is 50 ng/ml.

RESULTS

3.1 Urine samples

The results of the quantitative analysis indicate that no morphine or morphine-like synthetic substances were present in none of the twenty-two blank urine samples (data not shown).

Four subjects were positive for morphine, nevertheless its concentration, between 8 and 19.3 ng/ml, was under the cut-off value (i.e. Figure 3).

Refer Figure 3 here – A) Chromatogram of the analysed urine sample of subject 1; B) Ion rates (m/z) of morphine (429, 414, 236) present in the analysed urine sample of subject 1.

One subject was positive for both morphine and codeine. The first was present in a concentration under the cut-off value (12.2 ng/ml), while the latter was over the cut-off value (134.1 ng/ml). Three more subjects were also positive for codeine, in concentration of 224 ng/ml, 82 ng/ml and 70 ng/ml.

Lastly, four subjects were markedly positive for morphine, in concentration of 487 ng/ml, 208 ng/ml, 205 ng/ml and 204 ng/ml. It should be specified that none of the participants ever took opiates or illicit substances; hence, it is reasonable to infer that the detected morphine, even though in concentration under the cut-off value, derived from the consumption of the provided bread with poppy seeds.

3.2 Poppy seeds sample

Gas-chromatography analysis on poppy seeds, mechanically extracted from bread, revealed the presence of morphine in the poppy seeds sample itself, as shown in **Refer Figure 4 here:** Chromatogram of the qualitative analysis on poppy seeds; B) Ion rates (m/z) of morphine (429, 414, 236) present in poppy seeds sample.

DISCUSSION

The absence of opiates in all the twenty-two blank urine samples

confirms with no exception that the presence of morphine, even if in minimal quantity, can be only due to the consumption of bread with poppy seeds. However, the quantity of poppy seeds, their nutrition facts or the species and the origin of the plant are not indicated in the nutritional information label of commercial bread with poppy seeds.

It is well known that morphine, so as other opiates like codeine and thebaine, is contained in the juice extracted from *Papaver somniferum* pod, a typical plant of the Middle East.

Initially they believed that the alkaloid was present only in juice and not in poppy seeds. However, since the 90s it became known in scientific literature that morphine and codeine are contained also in *Papaver somniferum* seeds. To this purpose, many international studies developed experimental protocols to detect these analytes [61–72]. Specifically, both analyses on seeds and empirical experiments on biological samples (like urine, blood, saliva) of volunteers after poppy seeds consumption have been performed. In the second case, biological samples underwent immunoenzymatic screening assays and confirmatory GC/MS analyses. Both analyses turned positive for the analytes under study, which were present in concentration higher than the cut-off values. In summary, international scientific community has already demonstrated that the ingestion of food containing poppy seeds, or of the seeds themselves, results in the introduction of morphine and codeine in human organism. These substances are assimilated, metabolized and excreted via the canonical pathways and, from subject to subject, this may result in a marked positivity of GC/MS analysis.

The detection of opiates in food raised the problem even at European level. In fact, in 2014 the European Commission issued "Recommendation on good practices to prevent and to reduce the presence of opium alkaloids in poppy seeds and poppy seed products" (2014/662/UE), which was published on 10 September 2014 on the Official Journal of the European Union [73] and adopted in Italy by the Ministry of Agricultural, Food and Forestry Policies. The recommendation brought the attention of political and medical establishment on the concrete possibility to find opiates, in particular morphine and codeine, in food products. Their presence may depend on harvesting and sorting modalities

of poppy flowers. Specifically, if these processes are not properly done by following specific passages and quality checks, contamination with poppy pods and, thus, with the juice full of opiates, may occur. For this reason, the European Commission reports also the procedures to be adopted during cultivation, harvesting and storage of poppy plants destined for human consumption and the cooking temperatures of the various edible products, hoping for optimal controls and quality management by manufacturing and distribution companies. Clearly, these controls are not always performed in the appropriate manner and therefore unprocessed poppy seeds reach the consumers. Of course, the limited quantity of morphine and codeine in these seeds is not enough to cause dependence or habituation in human adults. However, their presence may represent a threat for infants and foetuses, whose xenobiotics detoxification pathways are still under development. Concerning the social and working aspect, the detection of opiates in adults that have never assumed these illicit substances can lead to undeserved repercussions at work and cause a reputational damage.

Conclusion: Bread with poppy seeds bought at the supermarket was administered to twenty-two healthy volunteers of both sexes (eight women and fourteen men), having different physical characteristics and lifestyles and aged between 25-73 years old, whose urines (blank urine before bread assumption and sample urine after intake) were tested with the classical chemical-analytical methods for opiates detection.

Analyses on blank urine samples confirmed that none of the volunteers took opioid medications or illicit substances. Instead, the analyses on urine samples collected after bread with poppy seeds consumption revealed the presence of morphine and codeine in some samples. In particular, these substances were present in variable concentrations among different samples and exceeded the cut-off value only in few cases. The inhomogeneous result may depend on the fact that, between 2015 (year of the case report) and 2020 (year of the research project), the considered bread underwent modifications of its nutritional properties. For this reason, the concentration of the analytes in some urine samples in the present study are lower than the cut-off value. Moreover, even differences in metabolism and

lifestyle among individuals may have strongly conditioned assimilation, metabolism and excretion of the analytes.

Analyses conducted in this study confirmed anyway that morphine detected in urine samples, even under the cut-off value, unequivocally derived from the consumption of food containing poppy seeds.

The ingestion of poppy seeds not properly processed according to the European food hygiene and safety regulations may result in the accidental assimilation of opiates, mainly morphine and codeine. The consequent detection of these substances during forensic toxicological analyses on workers puts their job placement, social position and family life at risk.

Eventually, this study leaves room for further analyses, since it cannot be excluded that the incidence of positive results for morphine may increase in case of a higher number of subjects, with different metabolic features, under study. Secondly, it should also be considered that a greater consumption of poppy seeds, regarding both absolute quantity and frequency, may result in higher morphine concentrations, even near or over the cut-off values established by law.

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Declaration of competing interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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Tables and Figures: IJMJ-2023-11



Figure 1 - Bread with poppy seeds bought at the supermarket.

Molecules	Retention Time (min)	Ion rate m/z
Morphine	16.00	429, 414, 236
6-MAM	16.53	399, 340, 287
Nalorphine	17.00	455, 414, 324
Scopolamine	14.58	375, 154, 138

Table 1 – Retention Time (RT) and Ion rate (m/z) of the considered molecules.

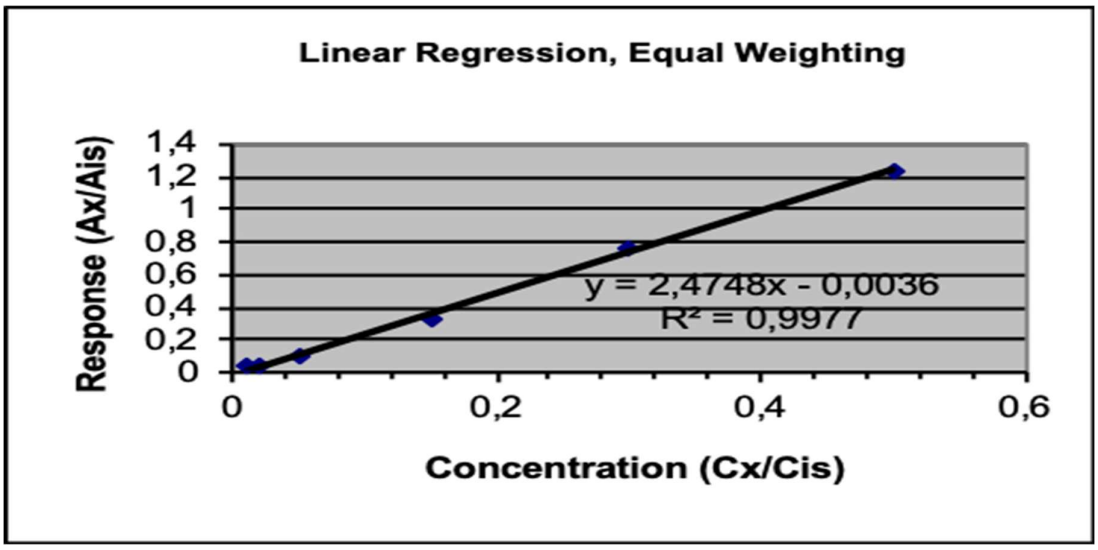
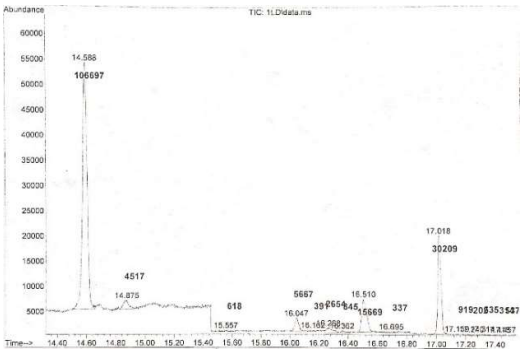


Figure 2 – The calibration curve and R^2 value.

A) 

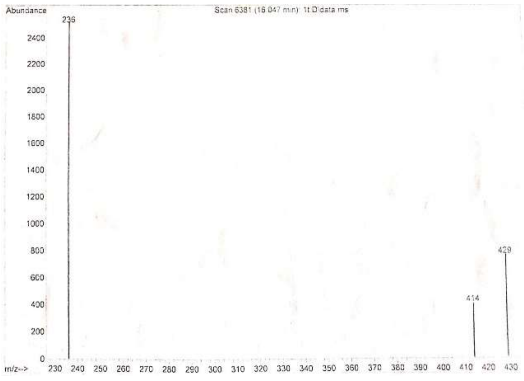
B) 

Figure 3 – A) Chromatogram of the analysed urine sample of subject 1; B) Ion rates (m/z) of morphine (429, 414, 236) present in the analysed urine sample of subject 1.

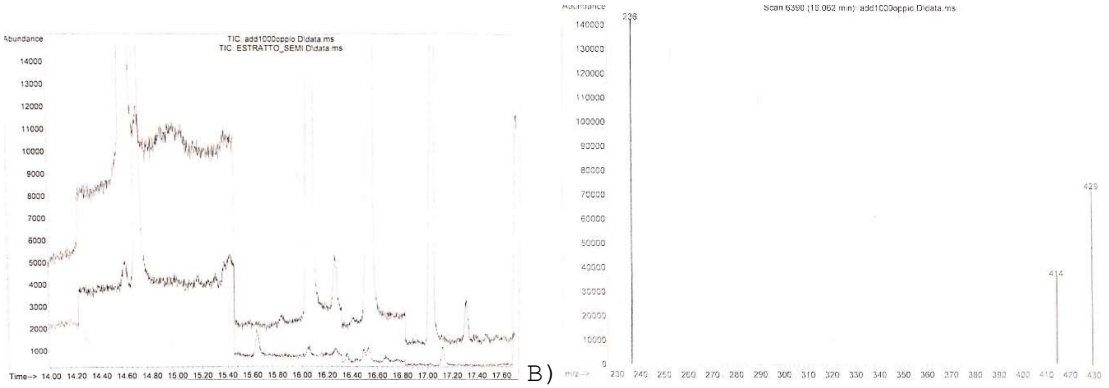


Figure 4 – A) Chromatogram of the qualitative analysis on poppy seeds; B) Ion rates (m/z) of morphine (429, 414, 236) present in poppy seeds sample.