

Abuse of illicit substances in two middle-north italian areas: study of prevalence using the same analytical methodology

L. Marchioro [°], P. Pezzati ^{*}, C. Fiore [°], G. Valentini [^], G. Biliotti ^{*}, P. Rizzotti [°]

[°] *Laboratory of Clinical Pathology, Geriatric Hospital ASL 16 Padua Italy*

^{*} *Clinical Laboratory, S. Maria Nuova ASL 10 Florence Italy*

[^] *U.O. Tecnologie e Procedure Informatiche ASL 10 Florence Italy*

Background. The Italian National Health Service has introduced, since 1990, a public service, called Ser.T. (Servizio per le Tossicodipendenze), devoted to the study, prevention and treatment of drug addiction. The service structures offer a unique opportunity to monitor the behavior of patients in remission, since urine analysis from patients unrolled in therapeutic programs is routinely performed.

Methods. In the present study, two clinical laboratories involved in Ser.T. activities, located in Center-Northern Italy cities, Padua and Florence, and sharing identical analytical protocols, compare the results obtained from urine toxicological screenings (homogeneous enzyme immunoassay, Emit II Dade Behring Inc. Cupertino, CA.).

Results. Some major behavioral difference are found: patients referring to the Florence Ser.T., have a higher percentage of positivity to opiates than patients followed in Padua (39.7% and 22.5 % respectively). A reverse situation appears when considering positivity to cocaine metabolites: 10.1% in Padua versus 0.86% in Florence. Florentine patients seem to be more prone to cannabis use (32.1% versus 18.8%), while the percentages of positivity to methadone are comparable (67% and 74% respectively).

As for benzodiazepines and amphetamines data, the methodological limitations of the urine screening test need to be taken in account when considering the rate of positivity found in Padua and Florence (27% and 19% respectively for benzodiazepines and 1.0% and 1.7% for amphetamines).

Conclusions. The shown variability in relapse may be due to several factors; the phenomenon of addiction in the course of the treatment needs to be further evaluated in order to define specifically designed protocols of initiatives in prevention and treatment.

Introduction

In recent years the use of illicit drugs, in Italy and in all developed countries as well proved to be an issue of crucial importance to all social institutions.

Reports of personal drug use have involved more than 100.000 young people in the period from 1990 to 1995. Only one-tenth of these cases (about 11.000) underwent special treatment programs. The system of public services and private rehabilitative structures shows a high acceptance by the users (n= 121.000) and a notable capacity for different interventions (1).

A detailed analysis of the different aspects of drug addiction, is recognized to be of paramount importance for all professionals involved in feasibility and efficacy studies, in health education and in therapeutic programs related to this phenomenon.

In 1990, in accordance with law 162/90 and subsequent legislation n° 444 of 09/30/90, a public service, part of the National Health Service, called Ser.T. (Servizio per le Tossicodipendenze, Service for

Drug Abuse), devoted to the study, prevention and treatment of drug abuse phenomenon, was introduced in Italy. Citizens seeking professional advice for any kind of addiction are encouraged to refer to the Ser.T. structures to be enrolled, as outpatients, in personalized programs. A team of different professionals (medical doctors, nurses, social workers and psychologists) works in strict collaboration to accomplish the complex task of helping the patient to return to a normal social life.

The care of Ser.T. for drug addicted patients involves several procedures intended to assess the level of addiction, to establish a therapeutic program and, finally, to verify the compliance to the therapeutic program. To accomplish the latter aim, samples of urine are collected, as part of routine activity, and tested for drugs and drugs metabolites.

Toxicology Laboratories as well as Clinical Laboratories to which these samples are sent, perform the analytical tests and support, with their expertise, the colleagues dealing with the patients. Moreover, laboratories may play a specific role in

the critical interpretation of data originated from the Ser.T. programs and in generating information of some relevance about the single patient's health, also from an epidemiological point of view.

Nevertheless, in order to evaluate data obtained from urine screening tests performed in drug abusers living in different geographical areas and sharing similar clinical history and treatments, the analytical methodologies used by laboratories need to be identical, to minimize the misclassification bias. This objective is very difficult to achieve on a national scale, given the number of involved laboratories and the variety of analytical protocols used, due to lack of specific regulation and poor compliance to the guide-lines on drug testing proposed by the Istituto Superiore di Sanità (2).

The present study compares urine screening results originated from two clinical laboratories, from January the 1st to June the 30th 1999. The laboratories involved are located in different geographical areas, namely Padua and Florence; they receive urine samples from patients enrolled in local Ser.T. programs and, notably, apply identical analytical methodology and share similar performance characteristics. The last features appeared to be an appropriate setting for a prevalence study, since the risk of misclassification bias, due to difference in analytical protocols, is reduced to a minimum.

Materials and Methods

Involved Structures

Laboratories

The involved laboratories in the study are: Clinical Pathology Laboratory, Geriatric Hospital ASL 16 Padua and Clinical Laboratory, S. Maria Nuova Hospital ASL 10 Florence. Both laboratories are public structures of the National Health Service in hospitals equipped, respectively, with 314 and 400 beds. The analytical activity is performed for hospital departments, in and out patients, and Ser.T. Both structures are provided with a L.I.S. (Laboratory Information Server) which has supplied the data reported in this study. The one located in Padua is called T-Lab (TEAM Data, Padua, Italy), the other one in Florence, is called Italab (Dianoema, Bologna, Italy) implemented by a software (Winlabonet produced by U.O. Programmi e Procedure Informatiche. ASL 10 Florence) specifically designed to manage Ser.T. related activities.

Ser.T.

All the Ser.Ts involved in the present study enrol out-patients only; in Padua, as well as in Florence, addicted patients enrolling in rehabilitation programs on a voluntary basis, account for two-thirds of the total. The remainder of patients are usually referred to Ser.T. programs by the judicial authority.

The data presented in this study are derived from both categories; data obtained from patients attending Ser.T. programs in prison are not included.

The ASL 16 Padua Ser.T. are organized in two structures indicated as Ser.T. 1 and Ser.T. 2 which differ slightly in terms of patient number and area of recruitment, counting the Ser.T. 1, 516 city dwellers and Ser.T. 2, 758 patients living in suburban areas and in surrounding villages.

The ASL 10 Florence Ser.T. are organized in twelve units in a Department on Drug Abuse. Three Ser.T. were selected to participate in the present study and are indicated as Ser.T. C, D, F.

Ser.T. D and C are located in the centre of Florence and are in charge of 457 and 336 patients respectively, while Ser.T. F is situated in a large suburban area, and assures specific treatment to 222 people.

The Ser.Ts. provide a treatment of drug-dependence based on assistance by a team of professionals, specially trained in this area of medicine to support patients during the detoxification stage and to assess the appropriate pharmacological therapy. The rehabilitation programs include psychotherapy, counselling and social support which are believed to be the heart of effective treatment and appear to be mandatory to prevent relapse.

Sample Handling

Urine samples are collected in Padua as well as in Florence, from all patients, regardless of pattern of drug abuse and choice of pharmacological treatment, in the Ser.T.'s consulting rooms exclusively and under Ser.T. personnel surveillance. Samples brought by patients are not accepted; the identification and integrity of urine samples is on nurses responsibility.

Biological samples are stored at 4°C degree and sent to the laboratories within 48 hours to be analyzed. Immunological screening tests are generally performed in the following 24-48 hours; samples are kept at a temperature of 4°C for at least a week and then discarded. In selected cases they are kept at a temperature of -20°C for further analysis, such as gas chromatography/mass spectrometry (GC/MS) but no result from GC/MS are included in the present study.

Sample Analysis

Both laboratories are equipped with the automatic analyzer Mega Meck (Meck; Darmstadt, Germany) applying a homogeneous enzyme immunoassay method (Emit II Syva Company Dade-Behring Inc., Cupertino, CA) intended for use in the qualitative and semiquantitative analysis of drugs in human urine. The list of substances of abuse assayed are: opioids, cocaine metabolite, cannabinoids, amphetamine-methamphetamine, methadone, benzodiazepines. All tests were performed according to the manufacture's procedures and at the recommended cut off: opioids 300 ng/mL, cocaine metabolite 300

ng/mL, cannabinoids 50 ng/mL, amphetamine 1000 ng/mL, methadone 300 ng/mL, benzodiazepines 200 ng/mL (3-30).

Both laboratories routinely run as an internal quality control, a drug free sample and a positive sample containing all the assayed molecules, in concentration above the cut off (Liquid Drug of Abuse Control, level 1 and level 3, Syva Company Dade-Behring Inc., Cupertino, CA). Finally, Padua and Florence participate to the Proficiency Testing Program (Center of Behavioral and Forensic Toxicology, Padua University), an external quality control program, specifically designed for drug of abuse testing.

Statistics

To compare the observed differences in proportions between Padua and Florence data, the chi-square test was used. The calculation of confidence limits (95%) of the results obtained was carried out with normal approximation of the binomial, while for their comparison, the chi-square (χ^2 proportion of independent samples) test was used (31).

Results and Discussion

As illustrated in Figure 1 and Table I, Ser.T. patients distribution does not show relevant difference between Padua and Florence. The large majority of people referring to such organizations is made of males, aged from 30 to 39 years, although in Padua Ser.T. the group over 39 shows a higher number of subjects. Table II summarizes the addiction typology: the most prevalent illegal substance abused (so called "primary abuse") by Ser.T. patients, is heroin. The Florence abuse distribution appears to be slightly different, although heroin

still represents the prevailing addiction problem.

With regards to pharmacological treatment, shown in Figure 2, methadone (an oral opioid agonist) protocols were the most common ones, as expected on the basis of primary abuse. Only a minority of patients were enrolled in naltrexone (a long acting opioid antagonist) protocols.

The procedure to address to the laboratory test requests vary between Padua and Florence Ser.T. The first ones may use defined profiles; Padua Ser.T. 1, in particular, uses either a so called "profile A" including: opioids, methadone, cocaine, or a "profile B" which contains besides opioids, methadone and cocaine, also amphetamine-methamphetamine and cannabinoids. Padua Ser.T. 2 may use the "profile B" and a further one, called "profile C" restricted to opioids and methadone. Both Ser.T, in some selec-

Figure 1. Patient Gender Distribution

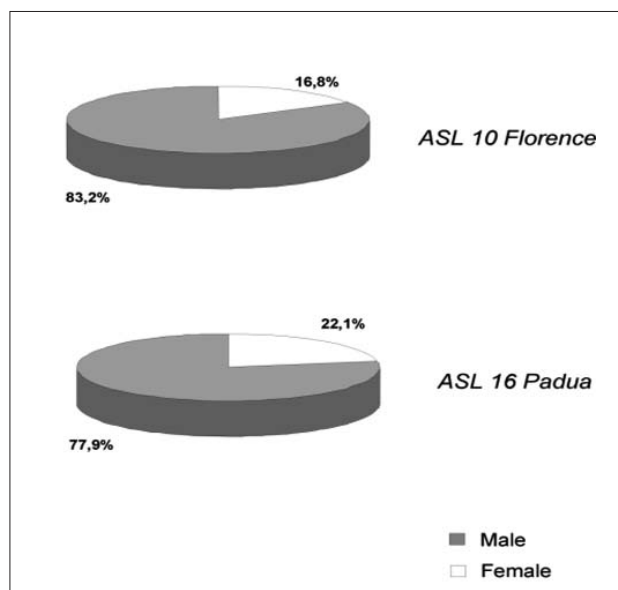


Table I. Age Patients Distribution

ASL 10 Florence	1-19	20-29	30-39	>39	Total
Ser.T. C	9 2,68%	123 36,61%	164 48,81%	40 11,90%	336
Ser.T. D	12 2,63%	181 39,61%	217 47,48%	47 10,28%	457
Ser.T. F	2 0,90%	90 40,54%	113 50,90%	17 7,66%	222
Total	23 2,27%	394 38,82%	494 48,67%	104 10,25%	1015

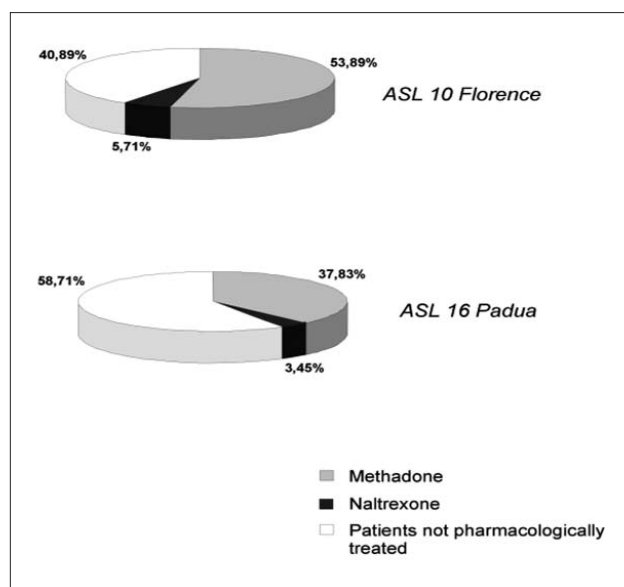
ASL 16 Padua	1-19	20-29	30-39	40-49	>49	Total
Ser.T. 1	14 2,71%	166 32,17%	252 48,84%	68 13,18%	16 3,10%	516
Ser.T. 2	29 3,83%	222 29,29%	391 51,58%	102 13,46%	14 1,85%	758
Total	43 3,38%	388 30,46%	643 50,47%	170 13,34%	30 2,35%	1274

Table II. Primary Abuse Distribution

ASL 10 Florence	Heroin	Cocaine	Amphetamine	THC	other drugs	Total Patients
Ser.T C	315 93,75%	7 2,08%	1 0,30%	12 3,57%	1 0,30%	336
Ser.T D	369 80,74%	14 3,06%	10 2,19%	59 12,91%	5 1,09%	457
Ser.T F	200 90,09%	11 4,95%	3 1,35%	8 3,60%	0 0,00%	222
Total	884 87,09%	32 3,15%	14 1,38%	79 7,78%	6 0,59%	1015

ASL 16 Padua	Heroin	Cocaine, Amphetamine, THC, other drugs	Total Patients
Ser.T 1	512 99,22%	4 0,78%	516
Ser.T 2	757 99,87%	1 0,13%	758
Total	1269 99,61%	4 0,31%	1274

Figure 2. Patients distribution according to pharmacological treatments



ted cases, may request urine screening for benzodiazepines and alcohol (data derived from the analysis of this latter substance are not included in the present study). The demand for alcohol, benzodiazepines and cannabinoids as unique analysis is rare, being less than 1% of the total requests and it is restricted to the few cases in which those substances represent the “primary abuse” molecule.

Florence Ser.T., on the contrary, does not use predefined panels, but organize the urine screening tests on the basis of patient clinical history and therapeutic program follow up.

However, detailed information on the sampling protocols applied by Ser.T. are not available.

Although the variance in urine analysis request may partially explain the difference in rate of positivity found between Padua and Florence Ser.T., we believe that the data shown reflect the difference

in local drug abuse reality.

The Padua and Florence laboratories performed respectively in the considered period, a total of 37.759 and 45.900 test.

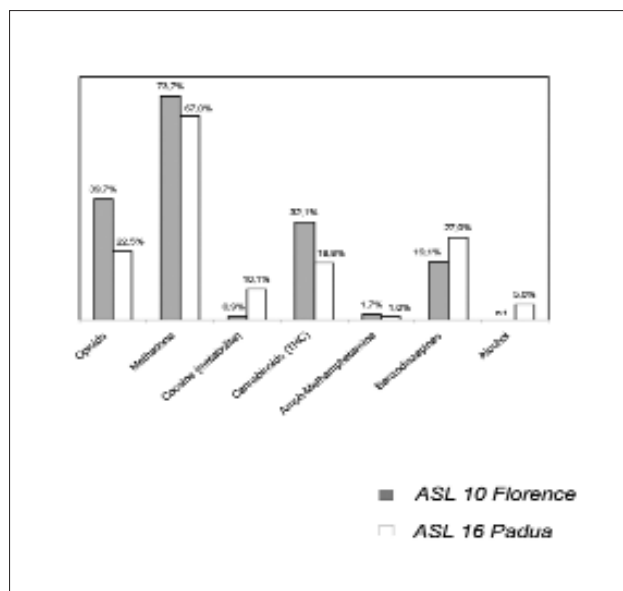
Table III and Figure 3 summarize the results of urine screening tests performed on samples from Padua and Florence Ser.T.; for each substance of abuse the positivity rate is shown; several observations may derive from such data. As previously mentioned, the majority of Padua and Florence Ser.T. patients have a clinical history of heroin addiction, in agreement with the national data (32); it is expected that such patients will be prone to some relapse. Padua subjects show a positivity for *opioids*, during the considered period of time, equal to 22.5% (20.5-24.5), while the rate of positivity for Florence patients is 39.7% ($\chi^2= 57.92$; $p<0.001$). We cannot exclude that such a significant difference may be influenced by the type of patients recruitment; the present study includes all patients referring to Ser.T., regardless of the beginning and the length of the treatment. Such parameters may determine the level of people compliance to the treatment itself and, consequently, the risk of relapse.

Interestingly, the rate of positivity found for *methadone* is quite similar for the two geographical areas: 67.0% (65.0-69.0) for Padua samples and 73.7% (69.7-77.7) for Florence samples, reflecting the uniformity of therapeutic choices due to the prevalence of heroin as molecule of primary abuse.

With regards to rates of *cocaine* positivity, the difference found between Padua and Florence is striking: urine samples collected from people enrolled in Padua Ser.T. reach the 10.1% (8.1-12.1) of positivity, in agreement with the national data (32), while samples from Florence Ser.T. are found positive in the 0.9% (0.1-1.7) of cases only ($\chi^2= 48.02$; $p<0.001$). Several factors may contribute to determine this result, e.g. the cocaine availability on the

Table III. Percentage of positive samples during Ser.T. treatment

	Ser.T. ASL 10	Ser.T. ASL 16
Opioids	39,7%	22,5%
Methadone	73,7%	67,0%
Cocaine (metabolite)	0,9%	10,1%
Cannabinoids (THC)	32,1%	18,8%
Amph-Methamphetamine	1,7%	1,0%
Benzodiazepines	19,1%	27,0%
Alcohol	not tested	5,0%

Figure 3. Percentage of positive samples during Ser.T. Treatment

illegal market with the economical and social environment of the drug addicts playing a major role, although many others may be hypothesized.

The misuse of *cannabinoids* appears to be a phenomenon more diffuse between the Florentine Ser.T patients than between people treated in Padua Ser.T. Urine samples obtained from the latter are positive to cannabinoids only in 18.8% (28.1-36.1) of cases, while Florence samples reach the significantly different percentage of 32.1% (28.1-36.1) ($\chi^2 = 21.57$; $p < 0.001$). For this particular substance of abuse, it seems legitimate to suppose that different general lines between the two geographical areas involved may exist, and, as a consequence, the episodic use of cannabinoids may be subject to different consequences, according to local rules. This factor, in addition to the others previously mentioned with regard to cocaine, may determine the frequency of cannabinoids abuse.

The *benzodiazepines* assays produced the following data: the Padua samples show a positivity of 27.0% (13.0-41.0) while the Florence ones show a positivity of 19.1% (14.1-24.1). The difference recognized does not reach a statistical significance ($\chi^2 = 0.81$; $p = n.s.$). Notably, the benzodiazepines screening tests requested by Padua Ser.T., during the period of time selected for the present study, refers only to known cases of primary abuse, which represent a minority of total

cases followed by the Ser.T. personnel; Florence Ser.T. are used to request benzodiazepines in a less selected population, in order to verify even the suspect of a therapeutic drug misuse.

Amphetamine and *metamphetamine* derivatives need a special consideration. Epidemiological studies (33) indicate that the number of amphetamines and derivatives users is steadily increasing in all European countries; nevertheless this kind of abuse seems to be scarce between drug addict people and rather appears to be a phenomenon of high relevance to the general young people population (34). The data derived from Padua and Florence Ser.T. urine samples seem to be agreed with those observations, since the percentages of positivity are for both Padua and Florence around 1.0%. It is necessary to underline the fact that a screening may not represent a valid method to assess the presence of amphetamine derivatives in urine. Molecules such as MDA (methylenedioxyamphetamine) or MDMA (methylenedioxyamphetamine, the so called "ecstasy"), the most common compound of the tablets on the illegal market, show only a weak cross reaction when tested using products designed to detect amphetamine-methamphetamine. The magnitude of cross reactions is usually declared by the manufacturer, but the risk of samples misclassification, due to the high incidence of false negative results, is a major concern to the laboratory personnel. It is possible to infer that a positive signal would be detectable in the presence of a high concentration of amphetamine derivative in the urine. Given the rapidity of MDA and MDMA metabolism such situation would take place only few hours after ingestion of a dose, making even more difficult for Ser.T. and laboratory personnel to reveal a urine positiveness. Although the pattern of amphetamine derivative diffusion as well as the psychological profile of people that assume such substances seems uncorrelated with Ser.T. patients characteristics, the availability of MDA and MDMA on the illegal market, may appeal to those Ser.T. patients less compliant and prone to relapse. A specific study designed to investigate the prevalence of amphetamine derivatives abuse in a Ser.T. population will bring interesting new information in this expanding field.

Conclusions

In the United States, as well as in Europe and Italy, prevalence studies referred to the diffusion, in the general population, of drug of abuse, are periodically conducted. In the last years, two relevant studies supported by the Federal Government, were published in the United States: the *National Household Survey on Drug Abuse* (NHSDA) and the *Monitoring The Future* (MTF). Although both surveys used valid type of prevalence measures, the results produced showed some inconsistencies, as pointed out by some Authors (35). The discrepan-

cies between the two prevalence studies originate from the difference in the study design which, as a consequence, affects the final results.

This paper presents a "period prevalence" study on drug abuse in a selected population (36). The subjects chosen are addicted patients enrolled in detoxification programs, clinically followed in specifically designed structures, called Ser.T.

The study shows the frequency of drug self administration during the treatment and compares the data obtained from two laboratories working in two different geographical areas, Padua and Florence. Both laboratories apply the same analytical methodology, with the advantage of reducing confounding factors such as differences in cut off.

Several conclusions can be drawn after examining the data obtained. From January 1999 to June 1999, 2289 urine samples obtained from patients enrolled in detoxification programs, were analyzed (55.6% by Padua Laboratory; 44.4% by Florence laboratory). Opioids appears to be the more frequently abused molecule even during the treatment, followed by cocaine and cannabinoids. Benzodiazepines and amphetamines are found only in a small percentage of urine samples. Interestingly, there are some statistically significant differences between relapse during treatment in Padua and Florence. In this latter area cocaine and cannabinoids play the major role either in relapse or in multiple abuse. In Padua, on the contrary, cocaine abuse is the major problem.

The presented data may be interpreted as the result of several factors. The variance in behavior between Padua and Florence drug addicts, may be caused by a different social and cultural distribution inside the Ser.T. considered. The illegal market, in addition, may strongly influence the choices of addicts. It cannot be excluded that Padua and Florence Ser.T. personnel may differ on cultural approach either to multiple abuse or to relapses, and may, consequently, take different actions.

All these observations contribute to define a very complex image of the addiction under treatment phenomenon. The variability of local situations appears evident and requires specifically designed initiatives, both in prevention and in treatment. In view of previous statements, laboratories collaborating in setting epidemiological studies, may contribute to the improvement of knowledge in the field of drug addiction and related aspects.

References

1. Avico U, Dell'Utri A. Drug Abuse Patterns and Trend in Italy. Community Epidemiology Work Group Meeting, New York 1996.
2. Zuccaro P, Pichini S, Altieri I, Pacifici R. Proposta di linee guida per l'analisi di sostanze d'abuso nei liquidi biologici. 1996, Rapporti ISTISAN 96/29: 1-26.
3. Urine testing for Drug of Abuse. National Institute on

- Drug Abuse (NIDA). Research Monograph 73, 1986.
4. Cox TC. Drugs and Drug Abuse. Addiction Research Foundation, 1983, 153-66.
5. AHFS Drug Information '87, American Society of Hospital Pharmacists, Inc. 1987: 1105-7.
6. Gorotezky CW. Detection of drugs of abuse in biological fluids. In Martin WR: Drug Addiction I. New York, Springer-Verlag, 1997: 319-409.
7. Oellerich M: Enzyme immunoassay in clinical chemistry: present status and trends. J Clin Biochem 1980; 18: 197-208.
8. Notice of mandatory guidelines for federal workplace drug testing programs: final guidelines. Federal Register 1988; 69: 11983.
9. Handbook of non-prescription drugs, Washington, DC, American Pharmaceutical Association, 1987; 142.
10. Baselt RC; Cravey RH. Disposition of Toxic Drugs and Chemicals in Man, 3rd ed. Chicago. Year Book Medical Publishers, Inc 1989, 320, 628, 738.
11. Diemk, Lentner C.(eds), Geigy Scientific Tables. 7th ed, Ardsley NY, Geigy Pharmaceuticals, 1975, 668.
12. Ellenhorn MJ, Barceloux DG: Medical Toxicology New York, Elsevier Science Publishing Company, Inc, 1988: 55-7.
13. Wyngarrden JB, Smith LH Jr (eds): Cecil Textbook of Medicine. Philadelphia, WB Saunders Co, 1988, 55-7.
14. Hayes LW, Krasselt WG, Mueggler PA. Concentrations of morphine and codeine in serum and urine after ingestion of poppy seeds. Clin Chem, 1987; 33/6: 806-8.
15. Pearman K: Cocaine: A review. J Laryngol Otol 1979, 93, 1191-9.
16. Stewart DT: Cocaine Metabolism: Cocaine and norcocaine hydrolysis by liver and serum esterases. Clin Pharmacol Ther 1978; 25: 464-8.
17. Hamilton HE, Wallace JE, Shimek EL Jr, LandP, Harris SC, Christenson JG. Cocaine and benzoylecgonine excretion in humans. J Forensic Sci Sas, 1977; 22: 697-707.
18. Van Dyke C, Byck R, Barash PG, Jatlow P. Urinary excretion of immunologically reactive metabolite(s) after intranasal administration of cocaine as followed by enzyme immunoassay. Clin Chem 1977; 23: 241-4.
19. Logan MJ. Quantitative determination of benzoilecgonine and cocaine in human biofluids by gas-liquid chromatography. Anal Chem 1977; 49: 1965-9.
20. Lindgren JE. Guide to the analysis of cocaine and its metabolites in biological material. J Ethnopharmacol 1981; 3: 337-51.
21. Woolf DS. Opioids, in Benett N, Voukaris C and Woolf DS (eds). Substances Abuse: Pharmacologic Developmental and Clinical Perspectives. New York, John Wiley and Sons. 1983: 70-85.
22. Hofman FE. A handbook on drug and alcohol abuse: the biomedical aspects, New York, Oxford University Press, 1983.
23. MC Burney LJ, Bobbie BA, Sepp LA: GC/MS and Emit analyses for Δ^9 -tetrahydrocannabinol metabolites in plasma and urine of human subjects. J Anal Toxicol 1986; 10: 56-64.
24. Fergeson J, Couri D. Electron-capture gas chromatography determination of benzodiazepines and metabolites. J Anal Toxicol, 1977; 1: 171-4.
25. Baselt RC, Steward CB, Franch SJ. Toxicological de-

- termination of benzodiazepines and metabolites. *J Anal Toxicol* 1977; 1: 10-3.
26. Bugge A. Quantitative high-performance liquid chromatography of diazepam and N-desmethyldiazepam of diazepam in blood. *J Chromatogr* 1976; 128:111-116.
 27. Wad N, Rosenmudh, Hanife E. A simplified quantitative method for the simultaneous determination of diazepam and its metabolites in serum by thin-layer chromatography. *J Chromatogr* 1976; 128: 231-4.
 28. Sun S. Fluorescence. TLC densitometric determination of diazepam and other 1,4-benzodiazepines. *Method Enzymol* 1982; 84: 490-515.
 29. Rubenstein KE. Homogeneous enzyme immunoassay today. *Scan J Immunol* 1978; 8 (suppl 7): 57-62
 30. Dixon R. Radioimmunoassay of benzodiazepines. *Method Enzymol* 1982; 84: 490-515.
 31. Colton T. *Statistica in Medicina*. Padova: Piccin, 1976.
 32. Magliochetti N. Rilevazione attività nel settore delle tossicodipendenze, anno 1997, in *Bollettino per le Farmacodipendenze e l'Alcolismo*, XXII (1) 1999.
 33. Esposito G. Osservatorio Europeo delle Droghe e delle Tossicodipendenze (OEDT) Relazione 1998 sul fenomeno droga nell'Unione Europea. *Sole 24 ore Sanità*, 16-22, febbraio 1999.
 34. Santi M, Bertolotti S, Borselli D, Giaccherini S, Mingione E. Ricerca "Dance": indagine sul consumo di ecstasy nelle principali discoteche di tendenza dell'area fiorentina nella stagione 1996; in *Bollettino per le Farmacodipendenze e l'Alcolismo*, XXI (3) 1998.
 35. Gfroerer J, Wright D, Kopstein. Prevalence of youth substances use: the impact of methodological differences between two national surveys. *Drug and Alcohol Dependence*, 1997; 4: 19-30.
 36. Hennekens CH, Buring JE. *Epidemiology in Medicine*. Sherry L. Mayrent ed. Little Brown & Co., Boston 1987.