Reprint from

10th Cologne Workshop On Dope Analysis 7th to 12th June 1992 - Proceedings -

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Sport und Buch Strauß, Köln, 1993

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In: M. Donike, H. Geyer, A. Gotzmann, U. Mareck-Engelke, S. Rauth (eds.) 10th Cologne Workshop On Dope Analysis 7th to 12th June 1992. Sport und Buch Strauß, Köln, (1993) 325-343

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The Experience of Antidoping Control at the XI Panamerican Games in Havana, Cuba

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Introduction

Controlling drug abuse in competing athletes has become a highly specialised, complex task which requires much expertise and the use of sophisticated testing procedures (1,2). The organising committees of international sporting events therefore look to accredited laboratories to carry out doping control tests. In the case of Regional Games, laboratories officially recognised by the International Olympic Committee (IOC) are considered essential for testing.

The IX Panamerican Games held in Caracas in 1983 were considered a milestone in regards to the introduction of highly reliable technology for drug testing. Disqualifications and withdrawals from competition ocurred when it became obvious the strong analytical capabilities available (3). Reliability in doping testing continued at the X Panamerican Games held in Indianapolis in 1987 (4), six athletes being disqualified during the full event (5).

The XI Panamerican Games for 1991 were planned to be held in Havana, Cuba. No accredited laboratory was available in that country nor in the Central American area. Therefore, the organising committee of the XI Pan-American Games (COPAN) and the Pan-American Sports Organisation (PASO) turned to the IOC for advise in finding the best solution. After studying different possibilities it was agreed to grant Temporary Accreditation for a Doping Control Laboratory to be set set up in Havana by the already experienced accreditted laboratory of Barcelona (Spain). Instead of making a development that would be of no help for the Region, the project proposed by the Barcelona laboratory was to include resources from two other laboratories of the area (Mexico and Cuba) in order that they could benefit of the interesting experience.

Background

The Panamerican Games were held in August 2-18th 1991. The protocol defining the constitution and functions of the XI Pan-American Games Doping Control Laboratory was signed on April 27, 1991 in Havana. Only three months were left for actual specific preparation.

In addition to the resources of the laboratory of Barcelona (Municipal Institute for Medical Research, IMIM), the other two laboratories involved in the actual single team that developed the full project were Laboratorios ABC Química Investigación y Análisis S.A., LABCQIA, (Mexico), and the Centro Nacional de Investigación Científica, CNIC (Cuba). These two laboratories had different previous experience in Sport Drug detection techniques. Consequently, it was necessary to develop comprehensive Standard Operating Procedures and intensive training periods in order to harmonize the knowledge of the full staff. Training was provided for both Mexican and Cuban people by the Barcelona scientists in the LABCQIA facilities in Mexico D.F. in three consecutive periods.

The team from IMIM, Barcelona, was made up of 12 persons. The spanish analysts were responsible for all the laboratory procedures and analysis carried out. They brought disposable material, reagents, reference compounds, reference urines and small-scale instrumentation from Barcelona. The LABCQIA laboratory helped with the task of coordination, provided computer support systems, the main instrumentation, reagents and 12 people. The CNIC provided the facilities in which the laboratory was located, the logistic support and 15 people.

Administrative actions needed to be arranged prior to moving material into Havana were the agreement of suitable insurance contract and the obtention of export licenses and permits through respective governments. On July 16th, 1991, 12 tons (100 m^3) of instruments, laboratory equipment, reagents and material were flown into Havana by special plane from Mexico. At about the same time, material from Barcelona arrived in five different consignments. By July 21st, the laboratory had been completed and was manned by two specialists from Barcelona, three from Mexico, and members of the CNIC staff. The rest of the teams from Barcelona and Mexico arrived in Havana July 27th. After three days of intense preparation and last-minute details, the laboratory was ready to begin work on July 30.

Facilities

The laboratory facilities were located on the fourth floor of the CNIC. They covered a surface area of approximately 700 m^2. For the accomodation of the initial facilities to the standards required for the final setup, an implementation program was prepared, including moving benchplaces, new gases installations, new internal electricity networks, installation of ancillary equipment, re-painting, new office furniture and so on. Critical dates for the end up of each of the steps was carefully monitored. A contingency plan involving the possibility of moving the laboratory to Mexico or Barcelona, was prepared but eventually not needed.

The requirements for electrical power were estimated around 100 Kwatts and were supplied by an independent line taken directly from high voltage connection. Additionally, an electrical emergency plant was made available for ensuring power continuing supply even if general electric shutdown. It was used mainly preventively when tropical storms were forecasted. Need for heat disipation were estimated around 150,000 BTU per hour in order to mantain a temperature below 20 °C. Condensation systems for mantaining low humidity levels (below 50%) were needed on a continuous basis inside the laboratory. Priority telephone and fax lines for national and international communication were also needed.

Access to the laboratory was controlled at all times by Security Forces. During the period in which the laboratory was in service, only laboratory staff and authorized members of the Medical Commission of the Games were allowed access to the facilities. Before and after the period in which the laboratory was in operation, it was open to visits. Some of the more relevant were those of the Presidents of the Republic of Cuba, of the IOC, of the PASO and of the United States Olympic Committee. Also the team doctors of all delegations were invited to visit the facilities.

Organization and Quality assurance

Setting up a Doping Control Laboratory, involving three teams of analysts that had not worked together before, operating round the clock (24 hours a day for 16 days), and providing results within 24 hours, necessitated accurate planning and organisation. Therefore, the structural organisation of the laboratory (see figure 1) and the definition of the functions and responsabilities of each person within the organisation, was clearly defined.

A computer program was designed and written for the event. This program was created to help in five different functions and to provide with the necessary control upon any step

during the whole analytical process from reception to final reporting. The program was written using the FOX-BASE database for a network of five microcomputers that was installed through the laboratory.
The five main workstations were separatedly located and gave the control of: reception, pharmaceutical information, distribution and pre-analysis, management (batch assignment, results entry, etc), and consulting and revising. The program soon showed to be essential in guiding people to work in the proper way. Every workstation had its own menus and functions available and people allowed to use them had their unique password.

Some quality control strategies were follow to ensure maximum reliability of results. Each analyst was trained to carry out only one procedure, avoiding possible confusions or mistakes. Aliquots for each analytical procedure were grouped in batches. Each person was responsible for the entire handling of the batch (up to the obtention of the final extract ready to be analyzed instrumentally). In this way the responsibility for each analysis was unique and the quality control chain easy to follow. Our experience suggests that this method optimize the whole quality and reproducibility of results.

Each operation in the lab (analytical method, reagent preparation, etc) was described in minute detail in a comprehensive unambiguous standard operating procedure (SOP) text. This written procedures were especially useful during the training period and the clearness of the text showed to be crucial. Together with the batch of aliquots, a sheet was delivered containing an "exhaustive flow diagram" of the task and an "incidents table". The flow diagram (figure 2) was a scheme of the whole standard operating procedure to follow with spaces to mark each step done and to annotate the batch numbers of the different reagents and standards used along it. The incidents table allow the analyst to write down any discrepancy with the procedure to follow or any observation which might account for an anomalous result. At the end of the sheet, the analyst annotate the day and time of ending the job and signed. An example of a "flow diagram" sheet is shown in figure 2.

Each batch contained control samples so that reproducibility could be followed and possible defective batches rejected. Instruments performance was controlled and recorded in appropriate books. Gas flows were recorded twice a day. Every change (inlet, septa, autotune, etc) was recorded and filed.

Analytical instruments and ancillary equipment

The main laboratory equipment was the following: five HP 5890 gas chromatographs joined to HP 5970 mass spectrometers, one HP 5890 gas chromatograph attached to an HP 5971 mass spectrometer, two HP 5890 gas chromatographs with a nitrogen-phosphorus detector, an HP 1050 liquid chromatograph with a variable wavelength UV detector, an HP 1090 liquid chromatograph equipped with a diode array detector and an HP 1000 Computer system. All these instruments were from Hewlett-Packard, Palo Alto, California, USA.

For immunological analysis, 4 TDX coupled to a Data Track system and one IMX system (all from Abbott Laboratories, Chicago,USA) were used. Additionally, a gamma counter (LKB model 1282) for radioimmunoassay was available in other location.

Analytical procedures

A total of 8 procedures were established.

Preanalysis: Measurement of pH and Specific gravity

Procedure 1: It was used to screen for the presence of nitrogen containing volatile substances excreted free in the urine, which includes most of the stimulants, some narcotics and local anaesthetics.
The urine was extracted with distilled diethylether at strongly alkaline pH and using the salting-out effect (anhydrous sodium sulphate). The ethereal extract was analyzed by gas chromatography (GC) with a nitrogen phosphorus specific detector (NPD) using a 5% phenyl-methylsilicone fused silica capillary column.
Isomeric ephedrines were not well resolved using the screening conditions. The identification of such compounds was done by GC-NPD using a special temperature program.

Procedure 2: It was used to screen for the presence of nitrogen containing non volatile compounds excreted in urine mainly as conjugates (glucuronide or sulphate conjugates). It includes narcotic analgesics and some stimulants and their hydroxilated metabolites.

After an enzymatic hydrolysis with ß-Glucuronidase from Helix pomatia, the urine was extracted at alkaline pH with a mixture of distilled diethylether and tert-butyl alcohol using the salting-out effect (anhydrous sodium sulphate). The organic phase was dried and derivatizated with MSTFA and MBTFA.

Derivatized extracts were analyzed by GC-MS in full-scan mode using a 5% phenyl-methylsilicone fused silica capillary column.

Procedure 3: It was used to detect the presence of pemoline and to quantify caffeine. The urine was extracted in alkaline media (pH 9-10) with a mixture of chloroform and isopropanol. The organic layer was evaporated to dryness, reconstituted in methanol and anlyzed by HPLC with UV detection (280 and 216 nm), using a high-speed reversed phase column and a methanol:water mobile phase with gradient elution.
The confirmation of pemoline was designed to be done by GC-MS analyzing the TMS derivative of its 2-hydroxy analog, 5-phenyl-oxazolidin-2,4-dione, obtained after an acidic hydrolysis of the alkaline ethereal extract of the sample.

Procedure 4: It was used to detect the presence of anabolic steroids and probenecid. It was divided in two fractions: compounds and/or metabolites excreted free in the urine (procedure 4A) and those excreted in both free and conjugated form (procedure 4B).

In procedure 4A, compounds were extracted with distilled diethylether at neutral pH and using the salting-out effect (sodium chloride). The ethereal extract was dried and subjected to a sequencial derivatization to obtain the O-TMS-N-HFB derivative of the stanozolol metabolite 3'-OH-stanozolol and the O-TMS derivative of the rest of compounds.

The derivatized extracts were analyzed by GC-MS in SIM (selected ion monitoring) mode, using a methylsilicone fused silica capillary column. Three characteristic ions were acquired for each derivative.

In procedure 4B, the sample was subjected to a purification step with XAD-2 and to an enzymatic hydrolysis with ß-Glucuronidase from Escherichia Coli before extraction with distilled diethylether at alkaline pH (pH 9-10). The ethereal extract was dried and derivatized with a mixture of MSTFA, ammonium iodide and dithioerytritol to obtain the O-TMS derivatives of the compounds. The derivatized extracts were analyzed by GC-MS in SIM mode, using a methylsilicone fused silica capillary column.

Procedure 5: It was used to detect the presence of diuretics and probenecid (6).

The samples were twice extracted with ethylacetate at alkaline pH (pH 9-10) and using the salting-out effect (sodium chloride). The combined organic layers were dryed, reconstituted in a mixture of acetonitrile:water and analyzed by HPLC with a diode arraydetector, using a high-speed reversed phase column and an ammonium acetate solution:acetonitrile mobile phase with gradient elution.

The identification of the peaks was carried out by its RRT and its UV spectrum.

The confirmation was designed to be done by GC-MS of the underivatized extract for canrenone, after silylation of the extract with MSTFA for amiloride or methylation with methyl iodide for the rest of compounds.

Procedure 6: It was used to screen for the presence of ß-blockers in urine. The same extraction, derivatization and GC-MS conditions as procedure 2 were used.

Procedure 7: This procedure includes the fluorescence polarization immunoassays (FPIA) used to screen for the presence of amphetamine like compounds, opiates, cannabinoids and cocaine metabolite.

Procedure 8: This procedure includes the microparticle enzyme immunoassay (MEIA) technique used to determine the levels of human chorionic gonadotropin (HCG). A second analysis of a presumptive positive result was designed to be done by a radioimmunoassay technique (Pharmacía).

Samples and reporting

The laboratory received 741 samples (71% from men and 21% from women) during the 16 days of the XI Panamerican Games. In addition of many days falling in the range 20-55 samples per day, there were three days with significant number of samples. In the heaviest day there were 114 samples (figure 3).

In general, the bulk of the samples arrived at the laboratory by night (67% arrived between 10 p.m. and 5 a.m.) and the remaining arrived between 2 p.m. and 8 p.m. (figure 3). Accordingly, two different menshifts were organized to adjust for the time of arrival and number of daily samples. Preliminary analysis was carried out from 5 p.m. to 5 a.m.; samples were extracted from 9 p.m. to 5 a.m. and from 7 a.m. to 3 p.m.; and instruments were in service 24 hours a day.

The samples belonged to athletes participating in 30 different sports. A total of 7000 screening test were performed. Procedures 1,3 and 7 were applied to all samples. Procedure 2 was carried out on suspected samples already analyzed by procedures 1 and 7. Procedure 4, 5 and 6 were applied to the samples from sports selected by the Medical Comission of the Games. Procedure 5 was also applied to samples with low specific gravity values or

abnormal alkaline pH (n=100). Procedure 8 was carried out in all samples from males where procedure 4 had to be applied.

Statistics

pH. Most of the samples (81%) showed values between pH 5.0 and 6.0, and 18% between 6.0 and 7.5. Only 3 samples showed values of over 8.0 and only 6 samples showed values of under 5.0. Samples with basic pH values were particularly tested for the presence of stimulants (more concentrated extracts) and diuretics.

Specific gravity. Seventy-six per cent of the urines had specific gravity values in the range 1.005-1.030 (Figure 4). Those samples with values lower than 1.005 (12.1 %) were studied for potential diuretic content but no substance of this group was found to be present.

Caffeine. Extremely low caffeine levels (< 0.5 ug/mL) were detected in 77 % of the samples (Figure 4). Only 2 % of them had values higher than 5 ug/mL. None of the urines exceded 8 ug/mL.

Ratio testosterone/epitestosterone. The general overview of the ratios obtained is presented in Figure 4. A skewed distribution with a maximum about 1 was obtained. A second maximum involving a small number of samples was obtained in the range 4-6. Two of the samples had values slightly higher than 6 in the initial analysis but did not comply with the 95% confidence limit statistical criteria for declaring them higher than 6 when triplicate analysis were repeated (7).

Reporting

Results for samples non containing banned samples ("negative samples") were reported in writing to the Medical Commission on the 24 hours following the beginning of the analysis of those samples.

When a urine was found containing banned substances or their metabolites, a comprehensive written analytical report was presented. Subsequently, at the request of the Medical Commission, the counter analysis of the second split sample ("B" sample) was carried out in the presence of the affected athlete and other witnesses from the Medical Commission and the Delegation involved. In these cases, less than 48 hours elapsed between the reception of the initial urine sample at the laboratrory and the delivery of the

final report to the Executive Committee of the Pan-American Sports Organisation.Samples containing banned substances and reported to the Medical Comission of the Games are listed in Table 1. Some of the analytical results and metabolic schemes of them are reported in Figure 5, 6 and 7. Five of the positive results were blind control samples introduced at random by the Medical Commission to verify the proper functioning of the laboratory. The positive identification and interpretation of these results demostrated the reliability of the work carried out.

The Medical Commission considered true doping cases other five positive results and asked for a counteranalysis to be carried out. In all cases, the analysis of the "B" sample confirmed the initial results of the "A" samples.

Twenty-five female athletes declared they had taken contraceptive pills. 70% declared norethindrone based compounds. It should be noted that norethindrone produces a metabolite (19 nor-androsterone) that is also a metabolite of nandrolone, a banned anabolic steroid. Three samples in which extremely trace levels of 3α,5α-19-nor-androsterone were detected, were further tested for the presence of the main metabolite of norethindrone (3-hydroxytetra-hydro-19-norethindrone) that was shown to be present.

Additionally, 3 cases of non declared lidocaine and 1 case of marihuana consumption (all of them "restricted substances") were also informed to the Medical Comission.

General remark. The experience of the doping control during the XI Panamerican Games show the possibilities for international cooperation when a highly especialized analytical laboratory is needed. Usually the cooperation in analytical chemistry is established at the level of validating different laboratories (8), methodologies (9), testing reference materials (10), Proficiency Testing or Quality Assurance Programs (11-13). The experience reported here goes a step forward thus involving a real joint task developed by people, equipment and resources from different countries as a single team with a common goal. Cooperation to obtain instruments, facilities, reagents, materials and personnel should be a key point to carry out such a project. The planning needs to be careful as any aspect ranging from personnel (number and qualifications) to the smallest material, spare part or reagent needs to be taken into account.

Doping trends

The results obtained (5 real doping cases) confirmed the trend reported in Indianapolis (1987, X Panamerican Games) towards a reduction in the use of prohibited substances. After the unfortunate experience in Caracas (1983, IX Games) this is a cause for satisfaction. When comparing the number of positive samples with the number of total samples analyzed in the 1988 Seul Olympic Games, however, a similar percentage appears. Nevertheless, the results at the Games of the XXV Olympiad held in Barcelona in summer 1992 appear to confirm the decreasing tendency. In regards to the antidoping analysis in these Olympic Games, the experience here reported obtained at the XI Panamerican Games has been obviously be highly valuable for the analysts of the Barcelona antidoping laboratory.

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13. Davis KH, Hawks RL, Blanke RV. Assesment of laboratory quality in urine Drug Testing. A proficiency testing pilot study. JAMA 1988;260:1749-50.Table 1. Positive cases reported to the Medical Commission

Table 1. Positive cases reported to the Medical Commission

DOPING CLASS *	Nr OF CASES	SUBSTANCE REPORTED	GENERAL REMARKS	
stimulants	5	ephedrine	real	banned
		pseudoephedrine	real	banned
		cocaine	real	banned
		amphetamine	control	banned
		cathine	control	banned
narcotics	1	propoxiphene	real	banned
ß-blockers	2	acebutolol	real	banned
		propranolol	control	banned
anabolic steroids	2	metandienone	control	banned
		norethandrolone	control	banned
local anaesthetics	3	lidocaine	real	restricted
marijuana	1	THC	real	controlled

* According to the classification of the IOC International Olympic Charter Against Doping in Sport.

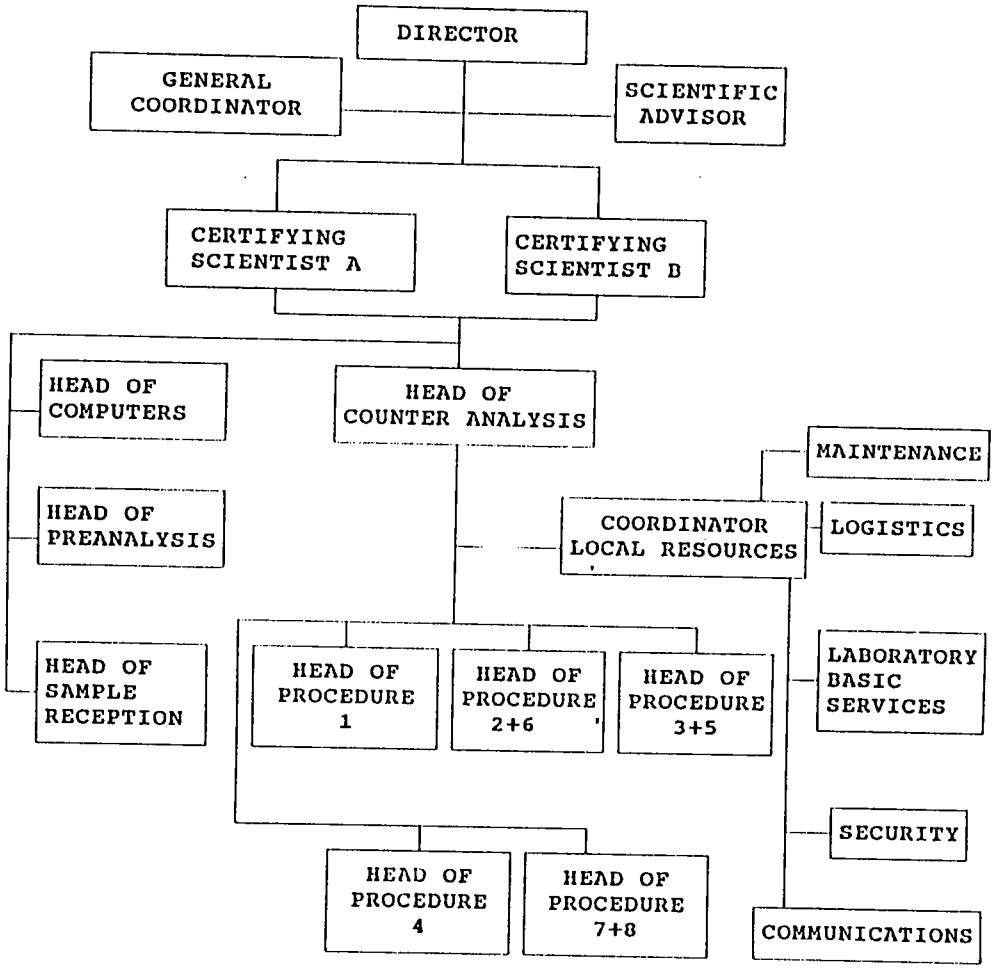
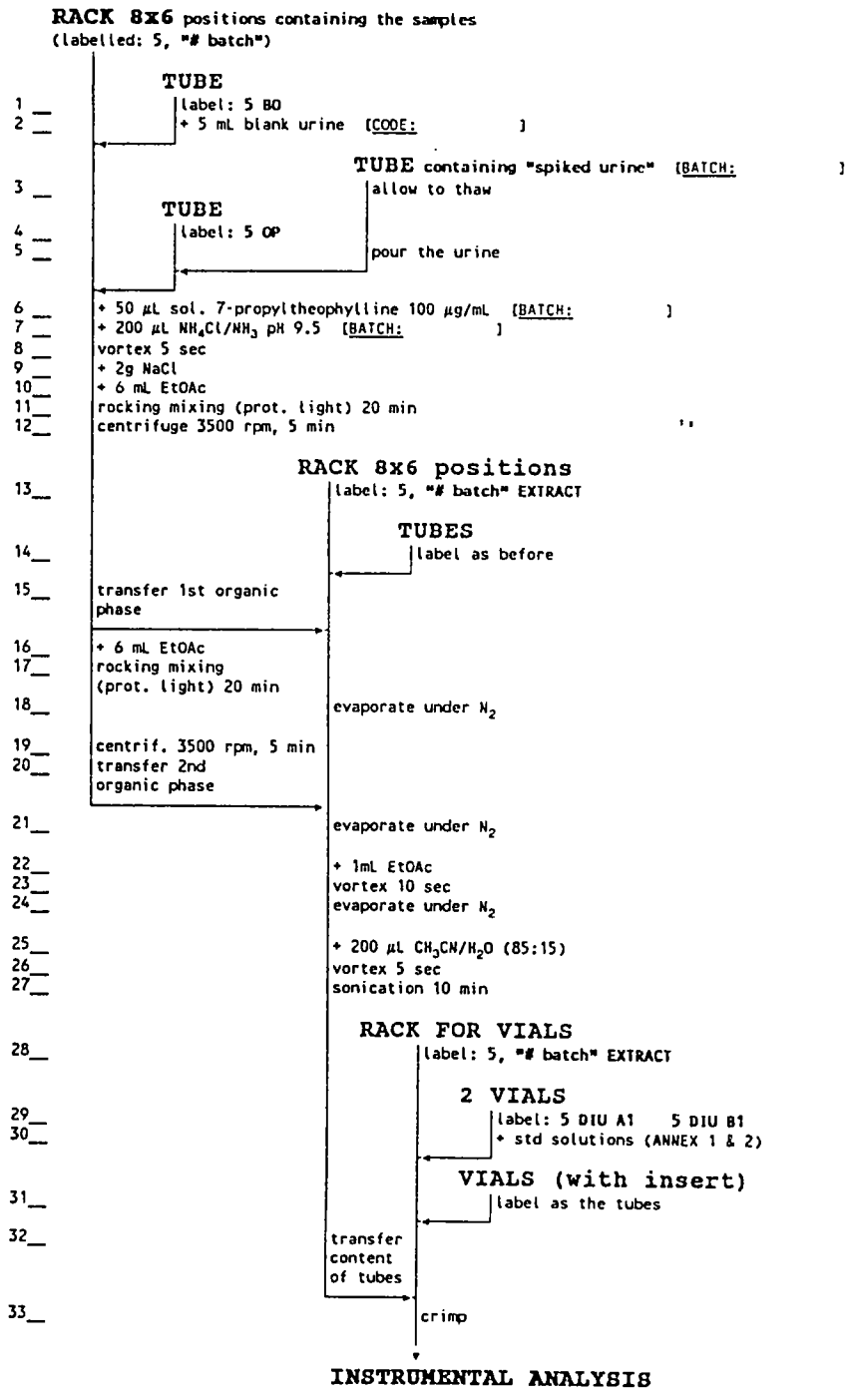


Figure 1.- General organization scheme of the tasks and responsibilities for the team working at the Doping Control Laboratory of the XI Pan American Games. Analysis of equestrian samples is not covered in this report.

INSTITUT MUNICIPAL	DEPARTMENT OF PHARMACOLOGY & TOXICOLOGY
D'INVESTIGACIO MEDICA	DOPING CONTROL LABORATORY

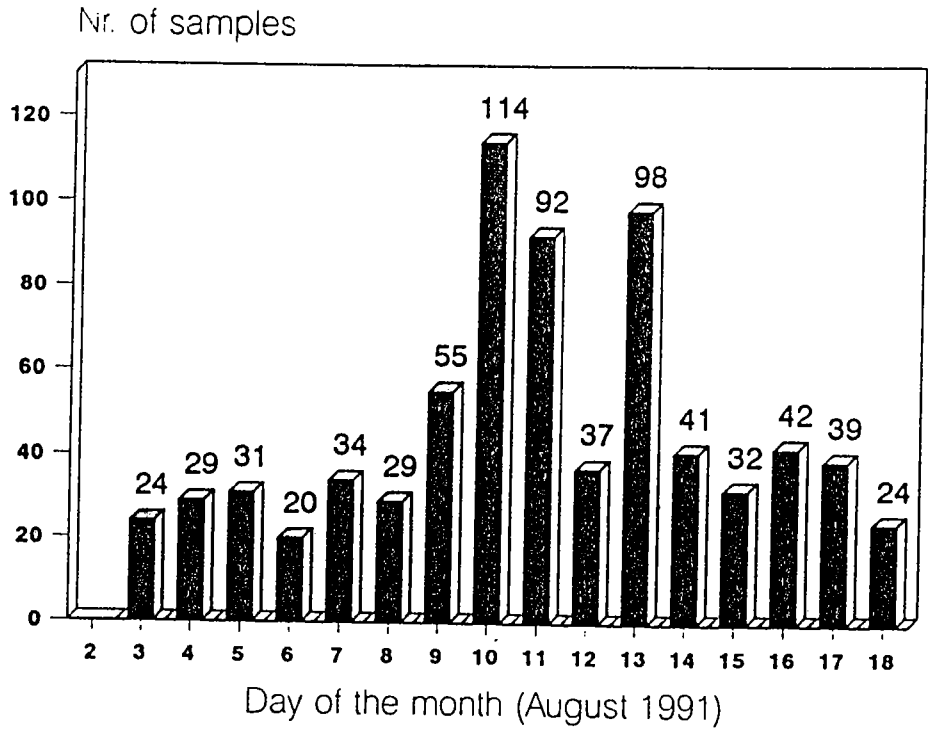
-- PROCEDURE 5 -- [SOP: MS004E02] BATCH Nr: _ _ _ _ _ _ _



DATE: NAME: SIGNATURE:

Figure 2.- Flow-diagram sheet received by analists involved in Procedure 1

DISTRIBUTION OF SAMPLES PER DAY



TIME OF RECEPTION OF SAMPLES

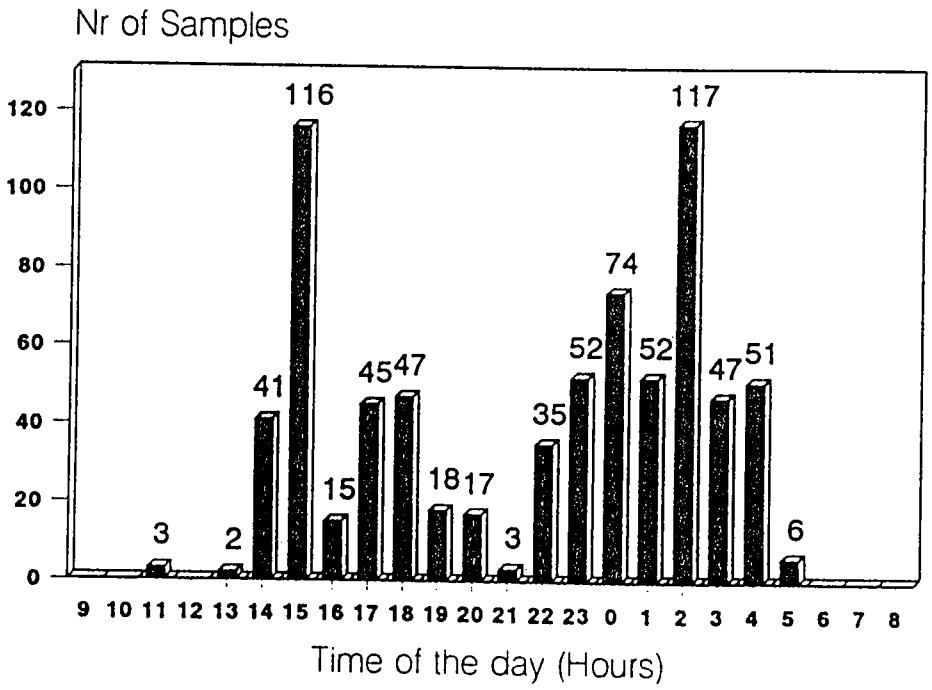


Figure 3.- Number of samples received grouped by a) day of competition and b) time of the day

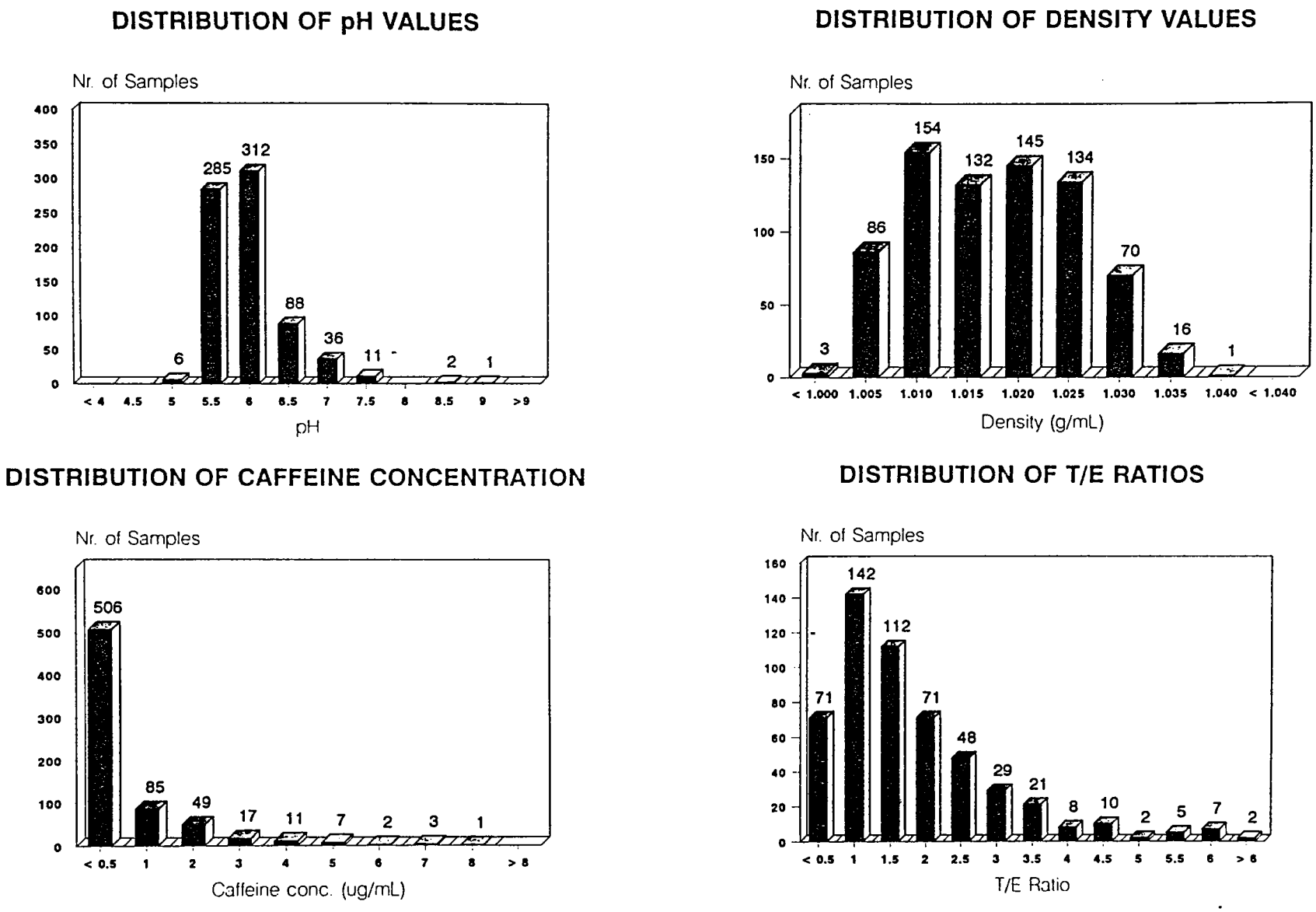


Figure 4.- Summary of the main quantitative results obtained for a) pH, b) specific gravity, c) caffeine and d) testosterone / epitestosterone ratio for the samples collected during the Games.

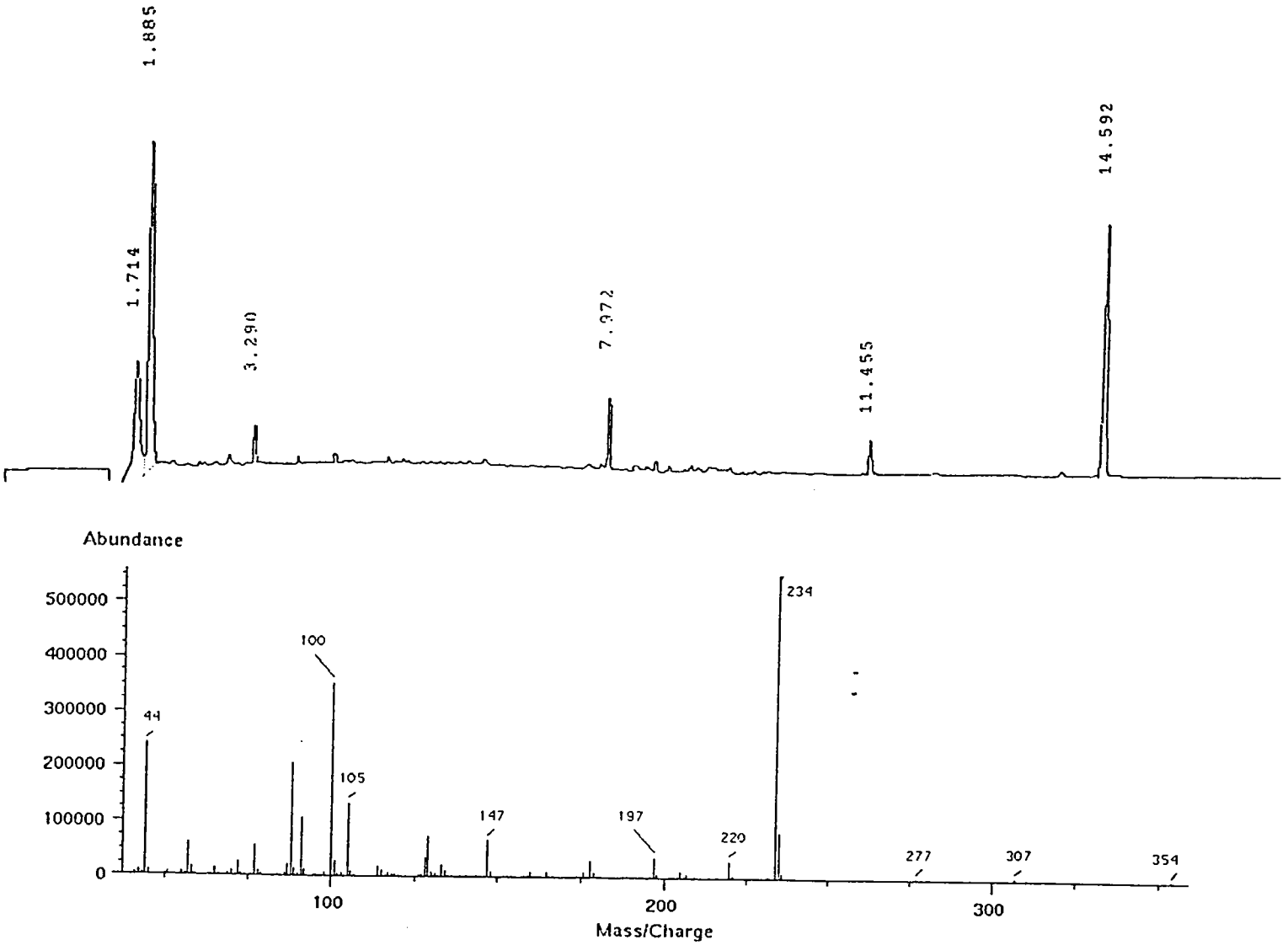


Figure 5.- Detection of nor-propoxyphene (retention time 14.6 minutes) by procedure 1 (top) and mass spectrum obtained for that peak (bottom).

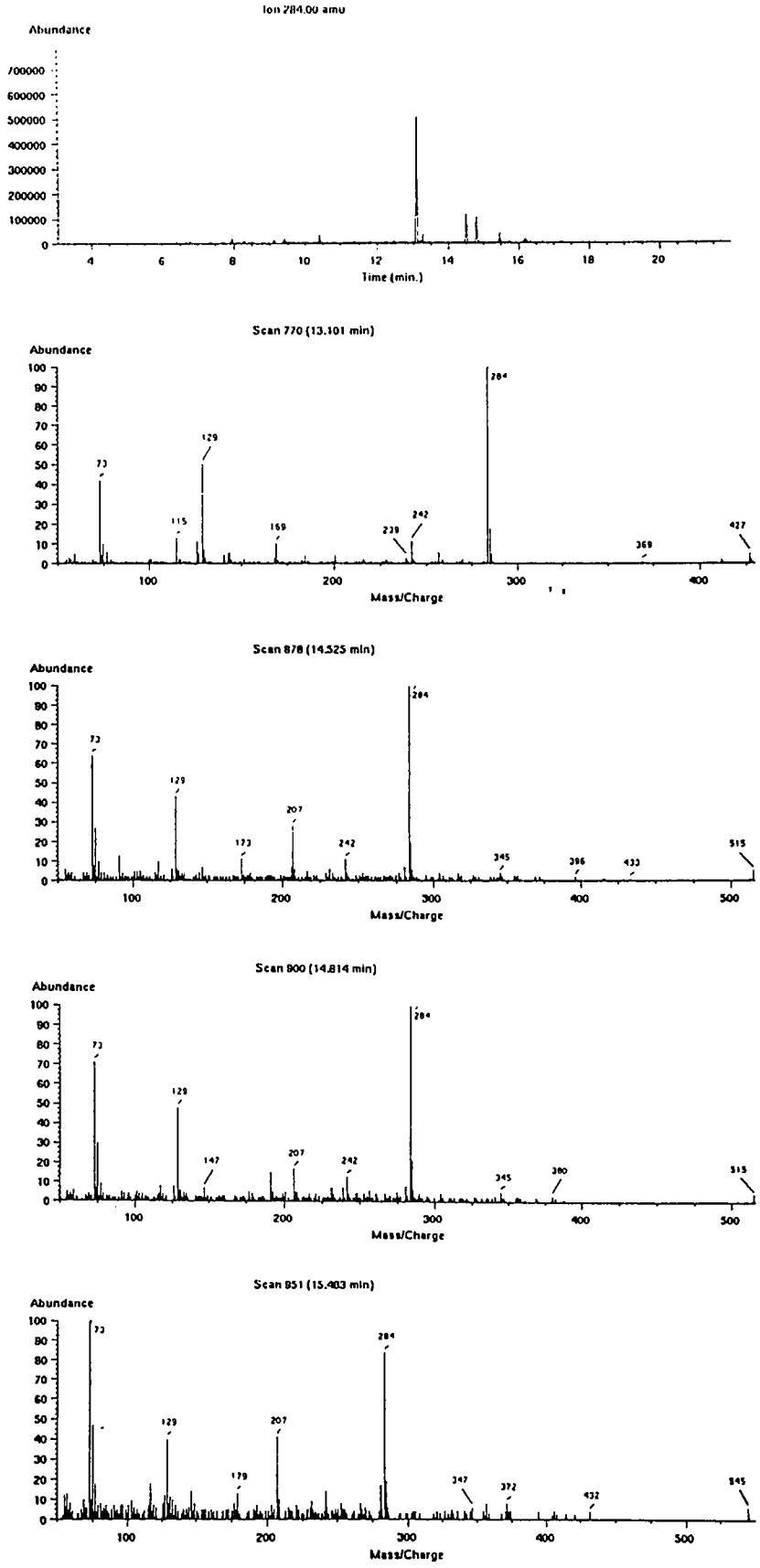


Figure 6.- Detection of propronolol and metabolites using procedure 2. From top to bottom: a) Mass chromatogram at m/z 284; b) Mass spectrum of the O-TMS-N-TFA derivative of propranolol; c) and d) Mass spectra of the bis-O-TMS-N-TFA derivatives of two hydroxylated metabolites; e) Mass spectrum of the bis-O-TMS-TFA derivative of hydroxy,methoxy-metabolite of propanolol.

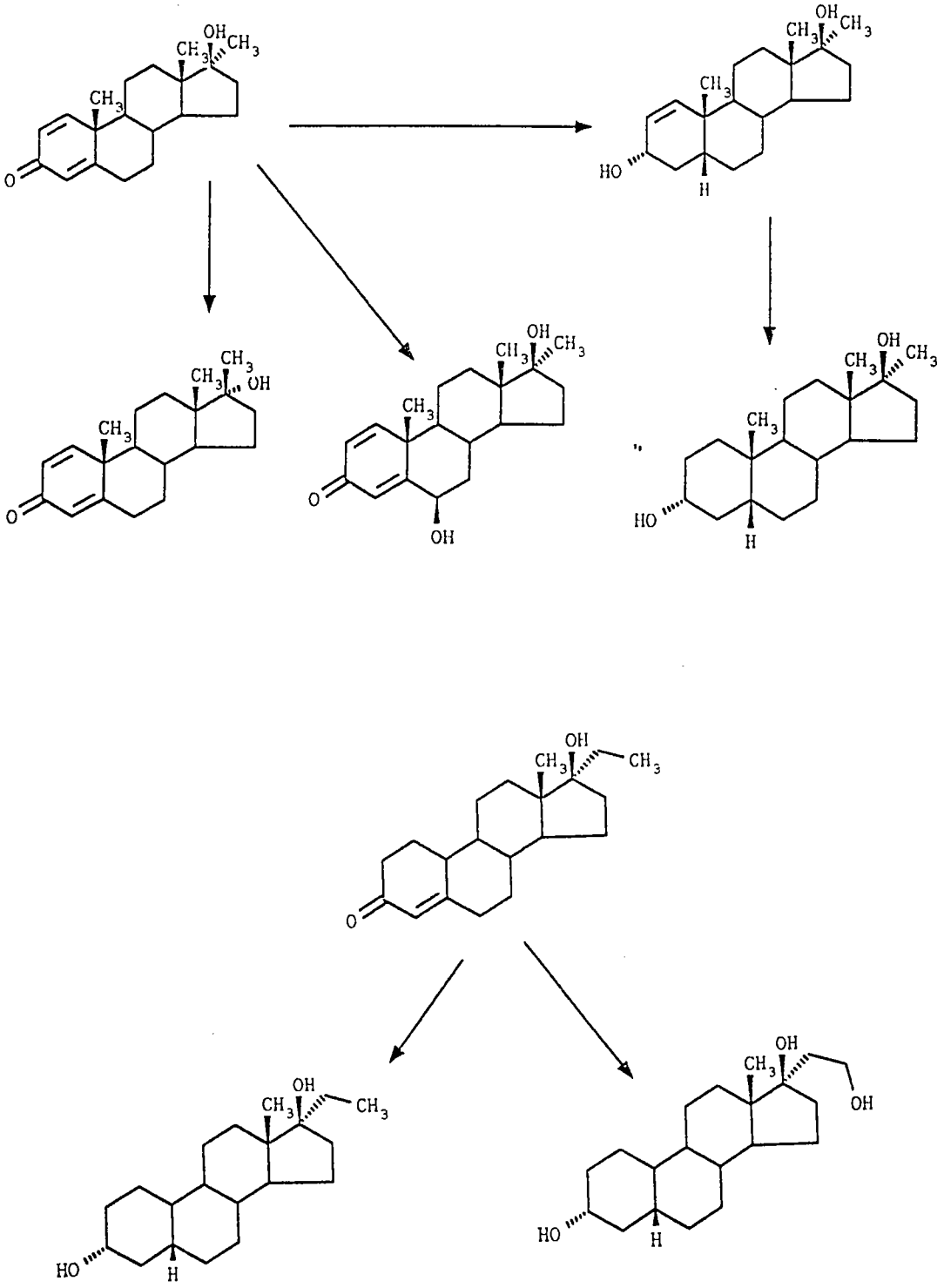


Figure 7.- Metabolic scheme for metandienone (top) and norethandrolone (bottom).