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Three cases of misleading results in drug analysis re-analyzed by GC-MS

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Abstract

This work shows three cases in which drug testing analysis were re-analyzed by GC-MS in order to confirm previous results. The drugs were Pentazocine, Delta 9-THC and Chlorphentermine.

It is difficult to find examples from the literature of drug testing or doping control with positive results, which were not reconfirmed later. This certainly deals with the different analytical procedures which are used.

The laboratories in developing countries , usually have not enough financial resources in order to have a GC-MS. In spite of the fact that the analysis are performed with the maximum available possibilities, these are normally not enough . There is no rules on what the minimum requirements and needs are for that type of laboratories on a National and even International level.

The tests were performed by different private laboratories in the first analysis and also in the confirmation analysis. Later on, one part of the sample was sent to us for additional confirmation. (Without any legal implication).

The laboratories involved were: one doping control laboratory and two drug testing labs. They have used different analytical techniques which are described and the additional confirmation analysis were performed at the Faculty of Chemistry. Finally, two cases were not re-confirmed although the legal penalties were applied to the suspected persons. The Delta 9-THC case, was positively re-confirmed by our laboratory.

1-Introduction

There are nine doping laboratories in South America. At present, four of them have been closed down, and left more than 12 GC-MS, 7 GC and 5 HPLC equipment unused. The remaining

laboratories are located in what is now called Mercosur (i.e. Southern America Common Market); one in Argentina, two in Brazil, one in Chile and one in Uruguay. This market involves an area of 12 million square kilometers with a population of more than 195 million people.
In South America there is no acreditable laboratory, although the whole area has 295 million inhabitants, and had represented more than 12 gold medals in Barcelona and 16 in Atlanta. These doping laboratories work with lack of support and equipment and although they try to do their best with the least, sometimes also they have failed.

It is difficult to find information in the literature about false positive results in doping analysis. On years of research we only found one publication with one false positive assay. This does not mean that they do not take place. It is only a fact that when they occur they are not published and in most cases they are hidden. This lack of information also contributes to the idea that doping analysis is very precise and has good reliability. The fact is that this is true if the analysis is performed in the proper way by suitable chemists with experience in the field. In order to give an example, one firm that produces GC-MS said in one of their advertisements that:'Depending on who performs the test and how it is controlled, some estimates claim that 5 to 20 % of screening test results are false'. Such information is written down and it is easy to imagine what lawyers could do if they take this type of information to a court.

2-Experimental

The first MS arrived in Uruguay in 1988 and the first lecture at the University about MS was given for the courses of Organic Chemistry III during 1989 academic year. By the middle of 1995 there were eight equipment in the country.
The three cases that are described involved three diferents private laboratories. The additional re-confirmation analysis were performed in the Faculty of Chemistry after the second analysis without any legal implication.

Case 1

In february 1995 the urine sample of a basketball player was found to contain Pentazocine (**I**). This drug is not commercially available in Uruguay and has never been detected in any other previous cases. For the screening analysis, the laboratory " case 1" followed the extraction procedure

described by R. Dugal, R. Massé et alt[1] for the screening I. One microliter of the ether solution was injected in a Shimadzu GC14B with a Shimadzu CR4 processor. Chromatographic conditions were: Column SE-54 , 30m, 0.22mm inside diameter x 0.25 micrometers of coating. The temperature of the injector was set to 300°C. The temperature was programmed at 10°C/min from 130°C not held to a final temperature of 300°C, maintained for 3 minutes. The Split/Splitless ratio was 1:10 and the injection was done with Grobe method for 45 seconds. The carrier was Hydrogen with a flow of 1ml/min. The detector was a FTD and diphenylamine was used as internal standard. The sample B was done in the same way by the same laboratory, with the same result. After the analysis of sample B, the sportman asked the court to send the latter to the university in order to be confirmed.

In order to control the analytical procedure,in the re-confirmation, we reproduced the extraction following the procedure described by R. Dugal, R. Massé et al[1]. For the analysis we used 0,2 microliter of the ether solution that was injected into the GC. The chromatographic conditions were: Column SE-54 , 30m, 0.22mm inside diameter x 0.25 micrometers of coating. The temperature of the injector was set to 300°C. The temperature was programmed at 10°C/min from 130°C not held to a final temperature of 300°C, maintained for 3 minutes. The Split/Splitless ratio was 1:20 and the inyection was done with Grobe method for 45 seconds. The carrier was Hydrogen with a flow of 1ml/min. The detector was a MS-Shimadzu QP2000, with ionization energy of 70eV. Codeine and Caffeine were used as internal standards.

As the result of this confirmation analysis was negative, we decided to reproduce the procedure of Jongsei Park[2]. For this procedure it was used a Column SE-54 , 30m, 0.22mm inside diameter x 0.25 micrometers of coating. The temperature of the injector was set to 280°C. The initial temperature was 120°C for two minutes and then programmed at 20°C/min to a final temperature of 300°C, maintained for 3 minutes. The Split/Splitless ratio was 1:20 and the injection was done with Grobe method for 45 seconds. The carrier was Hydrogen with a flow of 1ml/min. The detector was a MS-Shimadzu QP2000, with ionization energy of 70eV. Codeine and Caffeine were used as internal standards.

This procedure also gave negative results without any interference that could have induced false positive result. Taking into account the high sensitivity of the FTD detector we reproduced once more the second procedure with the double amount of urine and the same result was obtained.

Case 2

The private laboratory "case 2" was performing drug testing over the staff of one hospital. When they found Methamphetamine by TDX in the urine of one employee. The employee asked for a second analysis and it was conducted but the result was not confirm. Unfortunately, we were not allowed to received the information on the analytical procedures that were followed in both events. Nearly at the same time in another Drug Testing laboratory "case 2 bis" it was found a person positive of THC (**II**) by TDX. They did the confirmation analysis with the same method. Due to what has happened with laboratory "case 2", the laboratory "case 2 bis" sent the sample to the University for an additional confirmation for THC. This was done before the employee and the employer were informed of it.

For the additional confirmation we followed the extraction procedure of Prof. Dixit[3] and a practical modification in the detection by GC-MS of the THC using co-flow-elution technique was made.

The technique consisted of a normal GC-MS but the substance is trapped in the precolumn of the GC. After a certain time in which all the volatile substances have gone, it is injected 1 microliter of a polar solvent that will elute the delta 9-THC from the pre-column to the column. The time reproductibility after the second injection was 0.5 min. The advantage of this procedure is a really clean chromatogram and good mass spectra as a result. Another advantage is the fast way to confirm the presence of delta-9-THC in the free form. The analytical conditions were:

For the inmuno-analysis in the TDX equipment, 5 ml of urine were used in the normal conditions. For the confirmation the same procedure was performed. In both analysis had appeared THC with values over the blank. The result was postponed until the re-confirmation by GC-MS analysis was done.

For the re-confirmation we followed the extraction procedure described in reference [3].

3ml of urine and 300microliter of 10M potassium hydroxide solution were added to a large test tube. The mixture was vortexed and hydrolyzed for 15 min. al 60°C. The sample was cooled, and 165microliter of glacial acetic acid and 2 ml of a mixture of 100mM sodium acetate buffer (PH 7.0)-methanol (95:5) were added. The specimen was adjusted to pH 4.5-6.5 with glacial acetic acid and vortex-mixed. Bond Elut Certify II tubes were used. For the analysis we used 1,0 microliter of the solution that was injected in the GC.

The chromatographic conditions were: Precolumn BP-225, 1mt, 0.22mm inside diameter x 0.5 micrometers of coating, followed by a Column BP-5 , 25m, 0.22mm inside diameter x 0.5 micrometers of coating. The injection temperature was 300°C. The initial temperature was 250°C not held and then the temperature was programmed at 10°C/min to the final temperature of 300°C which was held 25 minutes. The split/splitless ration was 1:30, Grobe methode 45 seconds . The carrier was hydrogen at a flow of 1ml/min. After 15 minutes from the start, one microliter of methanol is injected. The detector used was an GC/MS-Shimadzu QP1100E with a ionization energy of 20eV and 70eV. Dipa-12 was used as internal standard.
Different SIM & TIC-Chromatograms were done, which confirmed the presence of delta 9-Tetrahydrocannabinol.

Case 3

The third case was on a horse racing event. A private laboratory performed the first analysis and found Fentermine (**III**). For the analysis they used 5 ml of urine which was extracted with chloroform. The pH to 9.00 was adjusted with a buffer of K_2CO_3-$NaHCO_3$. The solution was dried over sodium sulfate and evaporated at 80°C. The dry extract was dissolved in 40 microliter of chloroform. One microliter of the solution was injected into the GC. The chromatographic conditions were: Column DB-5 , 25m, 0.33mm inside diameter x 0.2 micrometers of coating. The temperature of the injector was set to 280°C. The temperature was programmed at 10°C/min from 120°C, initial time 2 minutes to a final temperature of 260°C, maintained for 3 minutes. The Split/Splitless ratio was 1:10. The carrier was nitrogen with a flow of 3ml/min. The detector was a FID-HP5890.

The confirmation was done with the extract obtained in the previous procedure. The extract was treated with 20 microliter of anhydride acetic and 2 microliters of pyridine. The mixture was heated during 15 minutes at 80°C. The solution was injected in the same conditions given for the first analysis.

In both analysis had appeared one peak which have had a similar retention time to the standard. The subtance was confirmed.

In order to control the analytical procedure, we reproduced the preparation of the extract and simultaneously we followed the procedure described by R. Dugal, R. Massé et al[1].
This procedure implies the preparation of two extracts, one from the free fraction and another from the conjugated fraction after an acid hydrolysis. The urine was extracted through a liquid-liquid procedure at pH=9.3, dried and rota-evaporated. The dry extracts were dissolved in 20 microliters of methanol with diphenylamine as internal standard.

The chromatographic conditions were: Column DB-1 , 25m, 0.33mm inside diameter x 0.2 micrometers of coating. The temperature of the injector was set to 280°C. The temperature was programmed at 10°C/min from 70°C, initial time 2 minutes to a final temperature of 300°C, maintained for 10 minutes. The Split/Splitless ratio was 1:50. The carrier was hydrogen with a flow of 1ml/min. The detector was a MS-Shimadzu QP1100EX, with ionization energy of 20eV and 70eV. Different SIM and TIC-chromatograms were done using the four fractions. None of them showed the same relative retention time of the standard. Finally we have looked for peaks which have similar retention times to the standard. Two peaks were selected and a library search after them was done. One of the unknown compounds can be proposed as vanillin with a 87% of similarity with the database and cresol with 97%. Solution of this substances were prepared and extracted with chloroform. The substrate was injected and the results has reconfirmed that those compounds could have been the interference in the original procedure.

3-Conclusion

It is easy to point out when something went wrong in one analysis but it is very difficult to realize meanwhile someone is doing it that something is going wrong. In the first part knowledge is needed but for the second experience and background knowledge is requiered. But there is a third part that normally is needed and forgotten that is: laboratory equipment. The joint of this three parts nearly garantee a good final analysis. There are laboratories that are named "doping laboratories" that not fullfill this requierements. In protecction of what it means we think that a guide shoud be written in which all the minimun requierements shoud be settled down. Not for a IOC acreditated laboratory just for the non acreditated doping laboratories.

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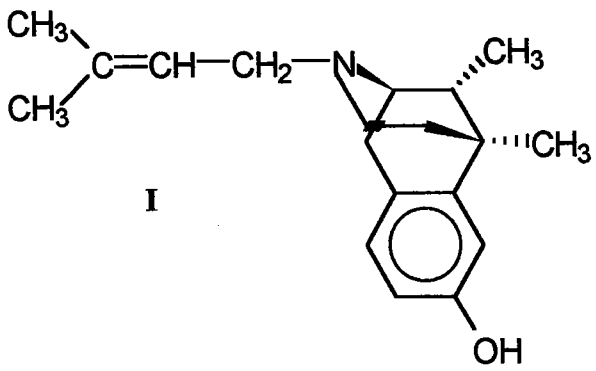
Figures:



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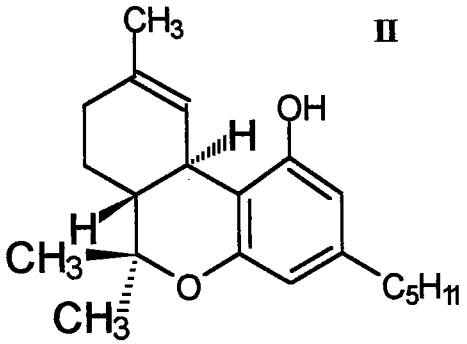

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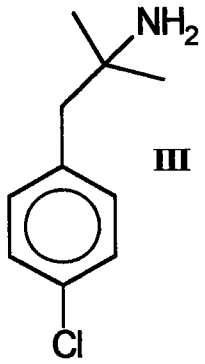

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