THE ANTIDOPING CONTROL IN HORSERACES IN ITALY

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ABSTRACT

The results and the improvement of the analytical procedures adopted for the control of doping in horses will be reported. This control has been systematically carried out in Italy for about 10 years in the laboratories of Italian Federation of Sport and Medicine in which the biological samples for the control of doping in various sport activities (football, cycling, athletics etc.) are also examined. In this way it is possible to use the same instruments for all these similar problems and compare the results.

The analytical procedure is based on the following steps:

- 1) Extraction of the samples (mainly urine but sometimes blood or saliva).
- 2) Screening tests by thin-layer chromatography.
- 3) Confirmatory tests by gas chromatography on different columns and also by gas chromatography coupled with mass spectrometry.

These single steps will be separately discussed, and practical problems encountered will be presented.

INTRODUCTION

In Italy, the antidoping analysis for horse races is carried out in the same laboratories as that for other sports such as cycling, football and track and field athletics. This has the following advantages:

- 1) The same analytical instruments can be used for all sports which means a better and more complete utilization of equipment.
- 2) Experience achieved in the analysis of new drugs in samples from man can be applied to the analysis of horse samples.

On the other hand, there is a large difference between the antidoping control of various sports and that in horse races. In athletics, for example, a list is published by the International Olympic Committee reporting the types of drugs which must not be used. Consequently we know during the analysis for which substances we are looking. In the antidoping control for horse races there is not, at the moment, a similar list, and consequently the analyst has to look for a very large and undefined number of substances. At present we have adopted an analytical procedure for the detection of the following groups of substances; barbiturates, stimulants, tranquilizers, alkaloids and narcotics (Cartoni *et al*, 1970).

Considering the large number of samples that must be examined in a short time, the analytical procedure is based on the following steps:

- 1) Extraction of the samples
- 2) Screening tests by thin-layer chromatography
- 3) Confirmation of identity by gas chromatography, mass spectrometry, etc.

EXTRACTION

Almost all the analysis is carried out on urine. Only in a few cases, when there are difficulties in obtaining a sample of horse urine, is saliva or blood examined. Urine is easy to obtain, and to use for drug extraction. The total amount of the drug is generally higher in urine than in other biological fluids.

Urine (20-50 ml) is made alkaline to pH = 9 with ammonium hydroxide and extracted with chloroform. The same sample is then adjusted to pH 11-12 and extracted again 2-3 times with chloroform.

Extraction curves of amphetamine and related substances (Fig. 1) show that compounds with only basic groups are not ionized in alkaline solution and can be extracted over a wide range of pH. For compounds containing acidic groups also, for example hydroxy-amphetamine, extraction is no longer possible above pH 10-11 because of ionization of the acidic group. Similar behaviour is observed for many other drugs.

To obtain a quantitative extraction for all these basic and amphoteric substances it is necessary to carry out

Figure 1. Extraction curves of some doping compounds.

1) benzamphetamine 2) fenfluramine 3) amphetamine 4) chlorophentamine 5) methamphetamine 6) p-hydroxyamphetamine 7) amphetamine.

the extraction at different pH as described above. The extracts are then combined, dried, and the solvent evaporated. The residue is dissolved in a small volume (0.1 ml) of methanol and used for the screening tests.

SCREENING

The screening procedure is based mainly on thin-layer chromatography (Cartoni *et al.*, 1968). For drugs like amphetamine and related compounds, the plates are coated with cellulose and are developed with n-butanol, formic acid and water in the ratio 20:1:2. The compounds are detected by spraying with bromocresol-green. The areas where the spots are located are scraped from the plate, and the drug extracted with ether. This extract is used for the confirmatory tests.

Screening tests for other drugs, such as alkaloids and tranquillizers, are carried out on silica gel plates with different solvent systems and methods of detections.

CONFIRMATORY TESTS

Following indications obtained from the screening procedures, gas chromatography is used widely for the confirmation of a suspected drug. Analysis is carried out of either a new sample of urine extracted as before or of the substance recovered from the thin-layer chromatogram, when a non-destructive spray reagent has been used.

Three types of columns are employed: SE-30, for the less volatile compounds, and Apiezon L and Carbowax 20 M. Retention indices are measured for the sample under analysis and these values compared with the ones obtained from reference substances (Table I). The reten-

tion index system is a very useful method for collecting and comparing data. Usually when gas chromatographic analysis is carried out on two columns of very different polarity and a perfect coincidence of retention indices obtained the identification of the drug is very satisfactory. The problem can be more complicated when the sample is found to contain a new substance with a retention index different from the available standards. In this case the combination of gas chromatography with mass spectrometry can be very useful.

This technique is now used either for confirmation of a drug or for the characterization of new products and metabolites. In our laboratory we have an A.E.I. mass spectrometer, Model MS-20, coupled with a gas chromatograph via a membrane separator. A large number of mass spectra of the more commonly used compounds have been collected and classified (Cartoni *et al.*, 1975). The classification is based on the system of the 10 most intense peaks, and each spectrum is arranged according to the increasing m/e value of the base peak.

CONCLUSION

At present, in our opinion, the antidoping control in horse races is very satisfactory, considering the modern analytical instruments available. When a sample is found positive for a given drug the substance can be identified with a high degree of confidence. The situation, on the other hand, is not so satisfactory for sample extraction and screening. There is the probability that some doped samples can escape detection either because the extraction procedure cannot be equally good for so many different compounds or because the screening methods are not sufficiently sensitive for all the drugs. Finally, sometimes an indication of doping is obtained during the screening procedure but identification of the substance is not confirmed because the amount of sample was too small.

TABLE I
Retention Indices of drugs used as doping agents
on various gas chromatography columns

Column	Molecular weight	Apiezon L		Type of Column Carbowax 20 M		SE-30	
Compound		T=148°C	T=162°C	T=152°C	T=165°C	T=135°C	T=180°C
Heptaminol	146	1090	1085	1735	1735		
Amphetamine	135	1147	1162	1675	1680		
Phentermine	149	1180	1195	1620	1635		
Methylamphetamine	130	1202	1210	1645	1650		
Ethylamphetamine	163	1230	1252	1645	1650		
Tranylcypromine	133	1235	1245	1880	1885		
Dimethylamphetamine	163	1260	1670	1660	1270		
Mephentermine	163	1275	1278	1690	1695		
Methoxymethylamphetamine	181	1345	1380	1895	1910		
Ephedrine	165	1370	1360	2150	2170	1360	
Pseudoephedrine	165	1370	1360	2155	2175	1360	
Phenmetrazine	177	1452	1455	2140	2145	1435	
Phendimetrazine	191	1470	1485	2035	2045	1435	
Prolintane	213	1620	1632	1990	2205	1605	1585
Fencamfamin	231	1677	1695	2170	2185	1655	1675
Methylphenidate	233	1685	1695			1705	1715
Caffeine							1805
Cocaine							2190
Codeine							2365
						T=210°C	T=240°C
Pipradol						2155	
Pemoline						2240	
Ethylmorphine							2450
Morphine							2485
Strychnine							3060

REFERENCES

- Cartoni, G. P., Cavalli, A. (1970) Analytical determination of doping agents. *Medicina dello Sport* **23**, 352-359.
- Cartoni, G. P., Cavalli, A. (1968) Detection of doping by thin layer and gas chromatography. *J. Chrom.* **37**, 158-166.
- Cartoni, G. P., Cavalli, A., Giarrusso, F. & Montanaro, M. (1975) Identificazione di sostanze doping con la gascromatografia e spettrometria di massa. *Medicina dello Sport* **26**, 15-22.

DISCUSSION

CHALMERS: Do you also have automatic spotting machines?

NAKAJIMA: We are trying to develop this, but at present it is done manually.

HAYWOOD: You say you have a method for detection of ethanol, do you have many positive samples?

NAKAJIMA: In 10 years we have had no positives, this is another reason we freeze samples during transportation to prevent formation of alcohols in urine after collection.