Reprint from

RECENT ADVANCES IN DOPING ANALYSIS (2)

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Sport und Buch Strauß, Köln, 1995

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In: M. Donike, H. Geyer, A. Gotzmann, U. Mareck-Engelke (eds.) Recent advances in doping analysis (2). Sport und Buch Strauß, Köln, (1995) 301-304

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Combination of screening procedures- Mesocarb detection as an example

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Introduction :

Depending on the chemistry and/ or biochemistry dope agents are detected in different screening procedures[1]. Polar substances and their conjugates present problems when an integration in existing screening procedures is intended. A typical example is mesocarb (3-(1-methyl-2-phenylethyl)-N-(phenylaminocarbonyl)-sydnon imine; "SYDNOCARB®"), a psycho stimulator and therefore a potential doping agent in sports[2]. The main metabolite excreted in human urine is a conjugated hydroxy-mesocarb (3-(1-methyl-2-phenylethyl)-N-(4-hydroxy-phenylaminocarbonyl)-sydnon imine). It should be possible to include hydroxy-mesocarb in the screening procedure for conjugated phenolalkylamines. But difficulties occurred detecting it after selective derivatization using trifluoroacetic acid as a solvent.
Mesocarb is thermolabile and undergoes pyrolysis in the injector block of a gas-chromatograph, and so does its metabolite. The pyrolysis product in both cases is N-nitroso-N-cyanomethyl-amphetamine which can be detected very sensitively with an appropriate GC/NPD/ or GC/MS/system.

This paper presents a method for the detection of mesocarb by combining two already existing screening procedures.

Experimental :

Instrumentation :

GC 1 :

HP 5880 with a NPD;

column :	SE 54; 0.35 µm filmthickness; 0.25 i.D.;
head pressure:	15 psi;
$T_{Injector}$:	280° C;
$T_{Detector}$:	300° C;
Temp.program :	100° C; 25°/min.; 300° C;3 min.

GC 2 :

HP 5890 with MSD 5971A;

column :	SE 54; 0.35 µm filmthickness; 0.25 i.D.;
head pressure:	15 psi;
$T_{Injector}$:	280° C;
$T_{Transfer\ line}$:	300° C;
Temp.program :	100° C; 23°/min.; 310° C;2 min.

Standards and chemicals:

N,N-Diisopropyl-dodecylamine (DIPA12) was synthesised in our laboratory;
β-glucuronidase from E. coli was purchased from BOEHRINGER-Mannheim;
XAD 2-resin was purchased from SERVA-Heidelberg ;
all other chemicals used were of analytical grade.

Hydrolysis and sample preparation :

2 ml aliquots of a Mesocarb excretion urine, obtained after application of 10 mg of mesocarb (24 h urine) were prepared in the following ways :

1. without hydrolysis
2. acidic hydrolysis
3. enzym. hydrolysis of the concentrated XAD 2 extract using β-glucuronidase from E. coli

Sample 2 was adjusted to pH 1-2 by adding 1 ml 6 M hydrochloric acid and then heated for 1 h at 100° C. Sample 3 was adjusted to pH 7 with 1 ml 0.2 mol/l phosphate buffer before adding 50 µl of the enzyme and then heated for 1 h at 50° C.

Sample 1 and Samples 2,3 after hydrolysis were adjusted to pH 9.6 by adding ca. 300 mg of solid carbonate/bicarbonate buffer (1:2/w:w). Then 2 µg of ISTD (DIPA 12) and 5 ml tert.-butyl-methylether were added, the samples were shaken for 20 min. and centrifuged. The organic layer was injected without further preparation.

Chromatography :

All samples were injected into a HP 5880 with NPD. For determination of the retention times of mesocarb and DIPA 12 (ISTD) a standard solution consisting of 50 µg mesocarb/ ml and 50 µg ISTD/ ml in methanol was used.

The retention time for the mesocarb was 4.19 min., for the ISTD 4.56 min..

The same solution was injected to a HP 5890 with a MSD 5971A to obtain the mass spectrum of mesocarb. The mass spectrum showed a base peak with m/z 91 and two small peaks with an intensity of about 5 % of the base peak with m/z 118 and 173.

Results :

The results of the different sample preparations are summarised in the following table:

	Type :	height[cnt]	height[cnt]	ratio	conc.[µg/ml]
		mesocarb/ OH-mesocarb	ISTD	(M/ISTD)	mesocarb/ OH-mesocarb
1	no hydrol.		15,54	0,00	0,0
2	acidic hydrol.	71,2	23,51	3,03	1,5
3	enzym. hydrol.(E.c.)	13,3	38,12	0,35	0,2
	STD mesocarb	84,74	40,78	2,08	1,0

Table 1: Yields of the different hydrolyses

The excretion study demonstrates that without any hydrolysis no mesocarb is extracted with tert.-butyl-methylether. The highest yield is obtained after acidic hydrolysis. After enzymatic hydrolysis using β-glucuronidase from E. coli mesocarb was detected with a concentration of about one eighth of the concentration of mesocarb after acidic hydrolysis.

The mass spectrum of the mesocarb peak is identical with the mass spectrum of a N-nitroso-N-cyanomethyl-amphetamine [3].

Mesocarb standard and the mesocarb metabolite give a signal at the same retention time and show mass spectra identical with N-nitroso-N-cyanomethyl-amphetamine.

Conclusions:

The expected concentrations of mesocarb after a 10 mg dose can be detected in an aliquot of the ether extract of the screening procedure for conjugated anabolic steroids. The procedure presented consists of a solid phase extraction using XAD 2 resin, enzymatic hydrolysis with β-glucuronidase from E. coli and finally a basic extraction with 5 ml tert.-butyl-methylether at pH 9.6. A 200 µl aliquot of the organic layer, which in our procedure is left in the centrifuge tube after decantation, is evaporated to dryness and reconstituted with 50 µl of methanol. 4 µl of this solution are then injected into a GC equipped with a NPD or a MSD.

Using this method even a ten fold dilution of the mesocarb excretion urine used in this study, which means a concentration of 0.2 mg/ml, gives a clear positive signal for mesocarb.

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[1] M. Donike et al; Dope Analysis; in P. Belotti et al; 1st International Athletic Foundation World Symposium on Doping in Sport, Florence 1987; International Athletic Foundation ; Florence (1988); 53-80

[2] M. Polgár et al; Metabolism of mesocarb in rat; Xenobiotica; 9; 1979; 8;511-520

[3] Communication to the IOC accredited laboratories; Nov. 1991