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W. Schänzer
H. Geyer
A. Gotzmann
U. Mareck
(Editors)

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Kress, A.; Mareck-Engelke, U.; Geyer, H.; Schänzer, W.

Alternative Bulk Materials to XAD-2 (Serdolit®AD-II)

Institute of Biochemistry, German Sport University Cologne, Germany

Introduction

The polystyrene divinylbenzene resin XAD-2 was used for the cleanup step in our sample preparation of anabolic steroids. Since this material with its particular quality is no longer available, we are looking for comparable and affordable alternatives.

Five bulk materials were tested under the same conditions as XAD-2 with regard to their handling and recovery: one polystyrene divinylbenzene copolymer (PAD-I) and four C_{18} solid phases.

Materials and Methods

The testing bulk materials are given below:

Polystyrene divinylbenzene resins:

- **XAD-2** –Serva, Serdolit®AD-II, particle size: 0.05-0.1 mm
- **PAD-I** – Serva, Serdolit®PAD I, particle size: 0.1-0.2 mm

C_{18} solid phases:

- Varian, Bondesil C_{18}, particle size: 0.04 mm
- Varian, Bondesil C_{18}, particle size: 0.120 mm
- Baker, Bonded Phase-C_{18}, particle size: 0.04 mm
- Macherey-Nagel, Chromabond C_{18}, particle size: 0.045 mm

Solid phase extraction (SPE):

For the conditioning of the adsorbent materials two different methods are needed. While the XAD-2 and the PAD-I resins are slurred in water, the preparation of the C_{18} adsorbents is performed with a mixture of water and methanol (1:1, v/v). Methanol is necessary to activate the C_{18} material.

Some preparation is according to the protocol:

2 ml of the blank urine were added to analytical grade Amberlite XAD-2 columns (pasteur pipette, XAD bed height ca 2 cm on glass bead – size: 3 mm or 1.5-2 mm, respectively {Fig.1}). The columns were washed with 2 ml of water and eluted with 2 ml of methanol [1]. 40µl of an internal standard solution [2] was added to the methanolic eluate. The mixture was evaporate to dryness.

The residue was dissolved in 1 ml of 0.2 M sodium phosphate buffer pH 7. To the buffer solution 50µl of *β*-glucuronidase from *E. coli* was added and the mixture was hydrolysed for 1 h at 50°C. The buffer solution was alkalised with 250 µl of $K_2CO_3/KHCO_3$ (1:1; 20%) to pH 9.6 and extracted with 5 ml of *t*-butyl methyl ether on a mechanical shaker for 5 minutes. After centrifugation the etheral layer was transferred and evaporated to dryness under vacuum.

The dry residue was derivatised with 100 µl of MSTFA/NH_4I/ethanethiol (1000:2:6; v:w:v) for 20 minutes at 60°C. 3 µl of the solution were injected to the GC/MS.

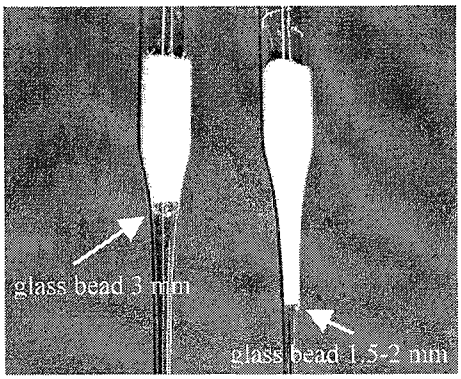

Fig.1: Analytically graded SPE columns closed with either 3 mm or 1.5-2 mm glass beads.

Capacity:

The capacity was tested only with the XAD-2 and PAD-I resins. 2, 4, 6, 8 and 10 ml of the blank urine were adsorbed on one column each. The further preparation steps follow the same procedure described above [1].

Direct hydrolysis (DH): the samples were prepared as described previously [2].

Results

Concerning the recovery of the endogenous steroids no significant differences were observed (Tab.1).

PAD-I columns, prepared with the normally used glass beads (3 mm), showed higher flow rates than the other tested adsorbents. Columns with smaller sized glass beads (1.5-2 mm) showed a strong reduction of the flow rates. This fact may lead to a more time-consuming sample preparation.

The handling concerning the column packing is comparable to XAD-2 only in case of PAD-I (Tab.1).

Finally the capacity of XAD-2 and PAD-I were tested and the results are shown in Tab.2 and Tab.3.

		polystyrene divinylbenzene			C_{18} solid phases			
manufacturer		Serva			Varian		Baker	Macherey-Nagel
	DH	**XAD-2**	**PAD-I**	**PAD-I**	**Bondesil C_{18}**	**Bondesil C_{18}**	**Bonded Phase-C_{18}**	**Chromabond C_{18}**
particle size [mm]		0.05-0.10	0.1-0.2	0.1-0.2	0.04	0.12	0.04	0.045
pore diameter [Å]					60	60	60	60
glass bead size [mm]		3	3	1.5-2	3	3	3	3
androsterone	2172	2308	2099	2203	2497	2581	2284	1936
etiocholanolone	1208	1283	1172	1242	1380	1422	1266	1094
testosterone	16	17	16	18	19	20	18	16
epitestosterone	29	33	31	33	35	37	33	29
11β-OH-androsterone	828	938	773	952	1011	1102	954	855
11β-OH-etiocholanolone	156	205	180	214	224	251	213	188
5α-androstane-3α,17β-diol	38	40	36	40	46	48	41	34
5β-androstane-3α,17β-diol	64	67	62	67	77	79	69	57
pregnanediol	169	171	167	172	201	188	185	128
conditioning		H_2O	H_2O	H_2O	H_2O/MeOH	H_2O/MeOH	H_2O/MeOH	H_2O/MeOH
handling		+	+	+	—	—	—	—

Tab.1: Comparison of the bulk materials PAD-I and C_{18} solid phases to XAD-2 with regard to their recovery and handling (DH: direct hydrolysis, concentration in [ng/ml]; +: good handling, —: bad handling).

	XAD-2				
quantity of preparation/column	**2 ml**	**4 ml**	**6 ml**	**8 ml**	**10 ml**
androsterone	2205	2142	2212	2167	2178
etiocholanolone	1226	1190	1204	1175	1184
testosterone	18	18	19	18	19
epitestosterone	36	35	37	37	38
11β-OH-androsterone	888	828	786	730	674
11β-OH-etiocholanolone	200	190	191	184	178
5α-androstane-3α,17β-diol	43	41	43	41	43
5β-androstane-3α,17β-diol	167	156	156	155	162
pregnanediol	150	153	162	158	166

Tab.2: Capacity of XAD-2 (concentration in [ng/ml]; number of sample preparation n=3).

	PAD-I				
quantity of preparation/column	**2 ml**	**4 ml**	**6 ml**	**8 ml**	**10 ml**
androsterone	2182	2131	2200	2061	2056
etiocholanolone	1256	1187	1201	1123	1109
testosterone	18	17	18	18	17
epitestosterone	36	35	37	36	36
11β-OH-androsterone	870	794	754	665	614
11β-OH-etiocholanolone	198	186	183	172	164
5α-androstane-3α,17β-diol	44	41	43	41	41
5β-androstane-3α,17β-diol	176	163	163	153	163
pregnanediol	161	160	171	163	164

Tab.3: Capacity of PAD-I (concentration in [ng/ml]; number of sample preparation n=3; columns closed with small glass beads {1.5-2 mm}).

Conclusions

PAD-I can be used as an alternative material to XAD-2. PAD-I is available as bulk material, significantly less expensive than commercially available C_{18} cartridges (Fig.2) and easily handled concerning manual column packing.

In order to achieve ideal flow rates, glass beads with a size between 1.5 and 3 mm should be tested. If those sizes are not available, the small sized glass beads (1.5-2 mm) should be used.

References

[1] Donike, M., Geyer, H., Gotzmann, A., Kraft, M., Mandel, F., Nolteernsting, E., Opfermann, G., Sigmund, G., Schänzer, W., Zimmermann, J.: Dope Analysis in: P. Bellotti, G. Benzi, A. Ljungqvist, (eds) Official Proceedings of the International Athletic Foundation World Symposium on Doping in Sport, Florenz 1987. International Athletic Foundation, Monte Carlo 1988, 53-80.

[2] Geyer, H., Schänzer, W., Mareck-Engelke, U., Nolteernting, E., Opfermann, G.: Screening Procedure for Anabolic Steroids – The Control of the Hydrolysis with Deuterated Androsterone Glucuronide and Studies with Direct Hydrolysis. In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck-Engelke (eds.) Recent advances in doping analysis (5). Sport und Buch Strauß, Köln 1997, 99-101.