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Analysis of Nutritional Supplements using Gel-Permeation as a Clean Up Step

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Introduction

Since the first analytical evidence of prohormone contaminations in nutritional supplements, many studies have been performed, confirming these analytical findings[1,2,3,4]. Strong indications lead to the conclusion, that in most cases the source of contamination is the manufacturing process, especially when contaminations at the low concentration range were found[5].

Although recently published methology[1,6], including extraction with MTBE or n-pentane under basic conditions and derivatisation with TMSI, enables to unambiguously identify prohormone contaminations less than 10 ng/g, it fails for about 10 % for the samples. Those samples (e.g. power bars) often contain a high content of fat and proteins.

Experimental

The presented method uses gel permeation as cleanup step after extraction of the supplement sample with cyclohexane. The method is summarized in Figure 1.

The following table presents gel permeation chromatographic (GPC) as well as gas chromatography - mass spectrometrical conditions.

Table 1: Instrumental conditions for gel permeation chomatography and GC-MS

GPC-Conditions:		**GC-MS Conditions**	
Column:	55 g Bio-beads SX3	**GC:**	Thermo Trace GC
Solvent:	ethyl acetate/	**MS:**	Thermo DSQ, IF at 280°C
	cyclohexane = 1/1	**Column:**	RTX-1, 15m, 0.1 mm, 0.1 µm
Flow:	5 ml/min	**T-Prog.:**	90°,40°min-1 to 170°,5°min-1 to 215°, - 320°
Dump time:	23 min	**Injection:**	1µl sl, Helium at 52 kPa
Collect time:	14 min	**Source:**	EI+, 70 eV at 250°C
Collection vol.:	70 ml	**Detection:**	Single Ion Monitoring

Results and discussion

Due to the very narrow molecular range of the analytes of interest (272 - 302), disturbing matrix contaminations can be separated from the chromatographical window of the subsequent GC-MS determination.

Although the method needs specialized instrumentation (low pressure GPC sample preparation unit), it allows to clean up supplements with high content of interfering material like fat and proteins. Figure 2 shows the efficiency of the GPC-clean up.

By using an extractive clean up without GPC, no prohormone can be identified due to the high matrix background (fat in this case), co-extracted with the analytes, present in a concentration of 100 ng/g supplement. After GPC-clean up, the internal standard (androsterone) is clearly visible and the matrix background is significantly reduced. The same supplement, spiked with 25 ng/g shows all signals of the target prohormons (see Table 2).

Till now, no type of supplement was found, which could not be analysed for prohormones.

Limits of detection expressed as a signal-to-noise ratio greater than 3 range from 0.02 to 0.05 µg/g (see Table 2) and are determined with three different nutritional supplements.

Table 2: Limits of detection determined with three different spiked nutritional supplements

Prohormone	LOD	Unit
4-Norandrostenedione	0,02	µg/g
4-Androstenediol	0,02	µg/g
4-Norandrostenediol	0,02	µg/g
5-Androstenediol	0,02	µg/g
5-Norandrostenediol	0,02	µg/g
Androstadienedione	0,05	µg/g
Androstenedione	0,02	µg/g
Epitestosterone	0,02	µg/g
Nandrolone	0,02	µg/g
Testosterone	0,02	µg/g
Dehydroepiandrosterone	0,02	µg/g
Metandienone	0,05	µg/g

Figure 1: Flow scheme of the entire method

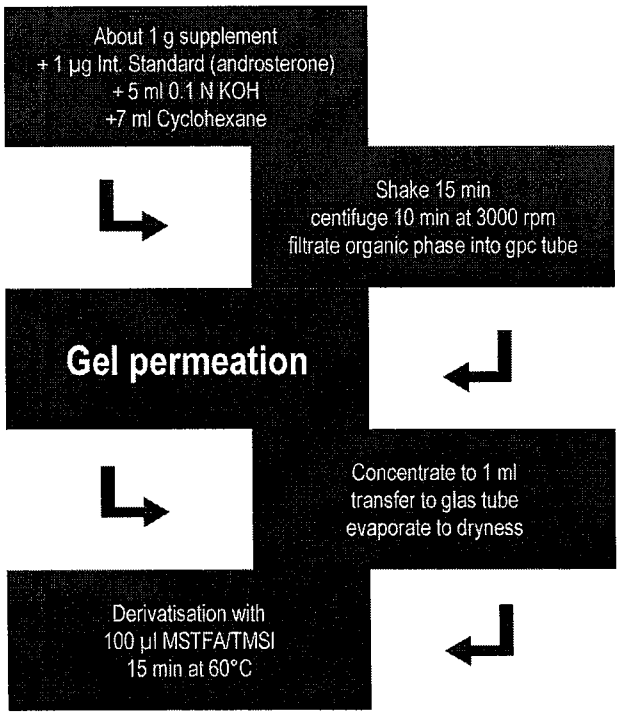
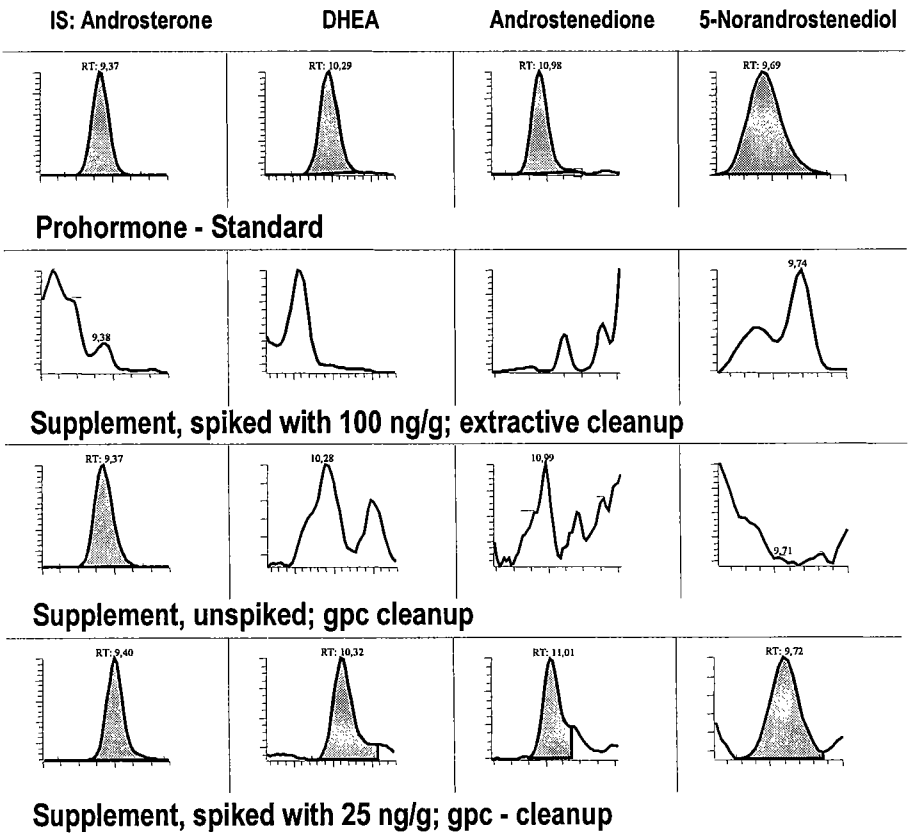


Figure 2: Comparison of extractive and gpc - clean up



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