XING Yanyi[1], LIU Xin[1], XU Youxuan[1] and WU Moutian[2]

Effect of different internal standards on steroids profile screening

1: National Anti-Doping Laboratory, China Anti-Doping Agency, Beijing, China

2: China Anti-Doping Agency, Beijing, China

Introduction

Athlete Biological Passport (ABP) is setting more and more important for doping control. Endogenous steroids in urine are of particular importance to ABP as they are the sensitive and retrospective biomarkers for anabolic steroid abuse by athletes. Many laboratories try to use deuterated internal standards to quantify steroids profile including T/E value, during screening and confirmation procedure [1-3]. From 2012, WADA will include endogenous steroids profile for assessment of laboratory performance in EQAS. Therefore, appropriate internal standards shall be included in the steroids screening procedure to obtain accurate semi-quantification result.

Experimental

All of the internal standards and the substances tested, which were accurately weighed, were mixed and dissolved in methanol. The mixed preparation and concentration of internal standard are summarized in table1-3. The methanol solutions dry with nitrogen. The residue was derivatized with 50μl of MSTFA/TMSI/ Ethanethiol (1000:3:2) at 70℃for 30 min and 1μl of the derivatized solution was injected into the GC/MS system.

GC/MS(Agilent 7890A/5975C) conditions:

GC-Injection port 280°C, interface:300°C , carrier gas:He, constant pressure:100kpa, column: HP-1,17m×0.2mm×0.11μm, temperature program: 180°C (3.3°C/min) 231°C (30°C /min) 310°C (2min), MS-Source temp 230°C, quad temp 150°C, SIM mode.

Table 1 *Sample 1-7 preparation*

(ng)	*1*	*2*	*3*	*4*	*5*	*6*	*7*
Testosterone(T)	25	50	100	150	200	250	500
Epitestosterone(epi-T)	25	50	100	150	200	250	500
5a-androstane-3α, 17β-diol	25	50	100	150	200	250	500
5β-androstane-3α, 17β-diol	25	50	100	150	200	250	500
Androsterone (An)	500	1000	2000	3000	4000	5000	10000
Etiocholanolone (Etio)	500	1000	2000	3000	4000	5000	10000

Table 2 *Sample 9-24 preparation*

Etio. Con. / *An. Con.*	*500 ng/ml*	*2500 ng/ml*	*5000 ng/ml*	*10000 ng/ml*
500 *ng/ml*	9	10	11	12
2500 *ng/ml*	13	14	15	16
5000 *ng/ml*	17	18	19	20
10000 *ng/ml*	21	22	23	24

Table 3 *Internal standard*

Internal standard	*Concentration (ng/ul)*	*Volume (ul)*	*Concentration in sample(ng/ml)*
d_3-testosterone(d_3-T)	10	5	50
d_3-epitestosterone(d_3-epi-T)	10	5	50
d_4-androsterone(d_4-An)	10	50	500
d_5-etiocholanolone(d_5-Etio)	10	50	500
d_3-5α-androstane-3α,17β-diol(d_3-5α-diol)	1	100	100
d_5-5β-androstane-3α,17β-diol(d_5-5β-diol)	1	100	100
Methyltestosterone(Me-t)	10	50	500

Result and Discussion

The statistics show that quantification results of T and epi-T with d_3-T and d_3-epi-T are more accurate than that with Me-t. Nevertheless deuterated internal standard is interfered endogenous steroids to some extent. When the concentration of endogenous steroids is less than 10 times of deuterated internal standards, the result is proximity to the reality value. If the concentration is 20 times of deuterated internal standards, the quantification results of T and epi-T are 15.5% and 13.5% low than real value (Fig1 and Fig 2). When the concentration of Etio is less than 10 times of internal standards, quantification of Etio with d_5-Etio is better than that with Me-t. If the concentration is 20 times of internal standards, the results are different-- 9.1% low than reality value by d_5-Etio, but 1.2% high than reality value by Me-t

(Fig 3). Using Me-t and d_4-An as internal standards to quantify An are both precise within 10 times concentration. When the concentration is 20 times of internal standard, the result is 5.3% and 21.5% low than real value by Me-t and by d_4-An separately (Fig 4).There was no difference between deuterated internal standard and Me-t to quantify 5α-androstane-3α,17β-diol and 5β-androstane-3α,17β-diol (Fig 5 and Fig 6) .

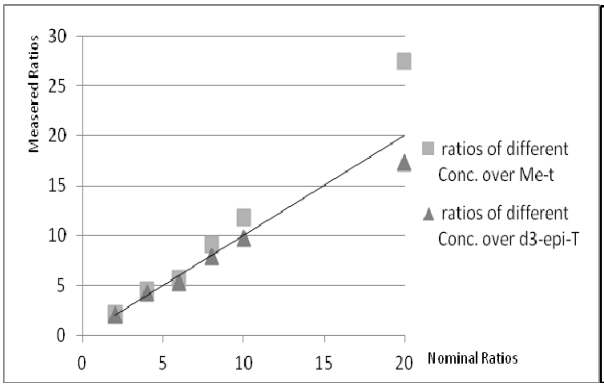

Fig1: quantification results of epi-T with d_3-epi-T and Me-t as internal standards

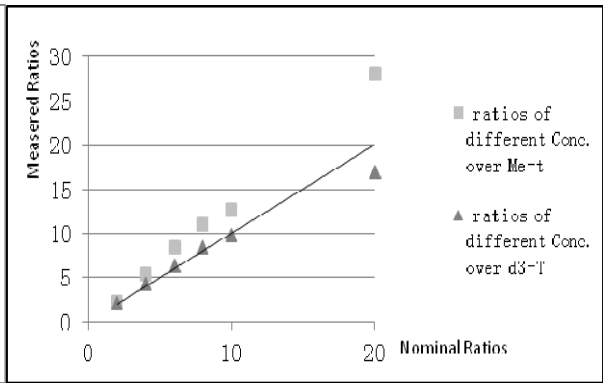

Fig2: quantification results of T with d_3-T and Me-t as internal standards

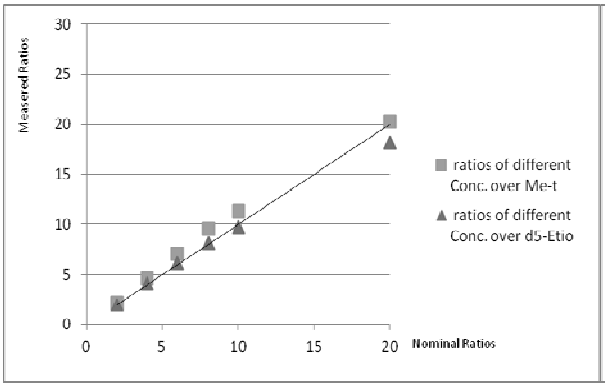

Fig3: quantification results of Etio with d_5- Etio and Me-t as internal standards

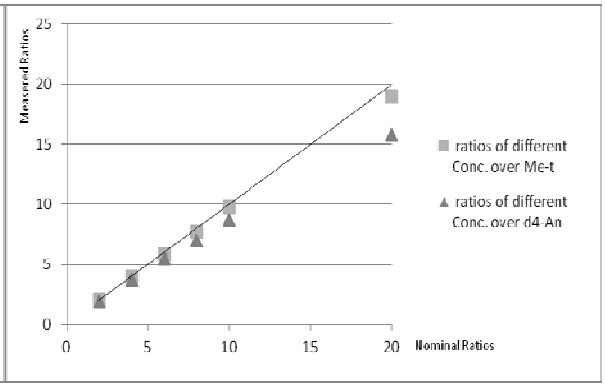

Fig4: quantification results of An with d_4-aAn and Me-t as internal standards

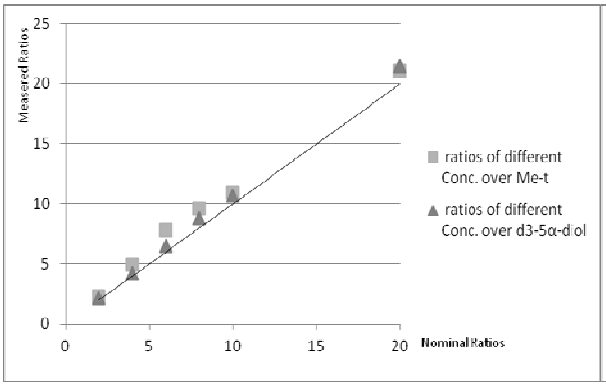

Fig5: quantification results of 5α-androstane-3α,17β-diol with d_3-5α-diol and Me-t as internal standards

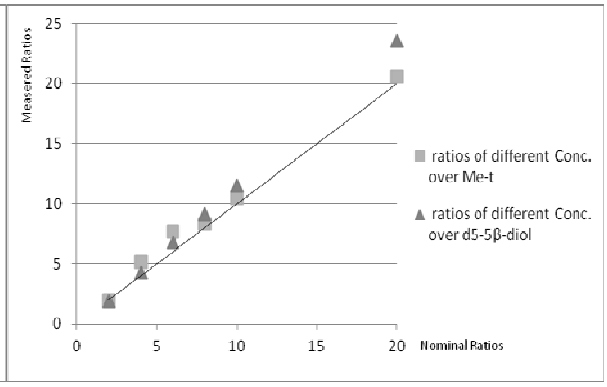

Fig6: quantification results of 5β-androstane-3α,17β-diol with d_5-5β -diol and Me-t as internal standards

When An and Etio exists at the same time, the effect on Me-t is greater than deuterated internal standards (Fig 7 and Fig 8). Hence it is more accurate to quantify An and Etio with deuteraterd internal standards.

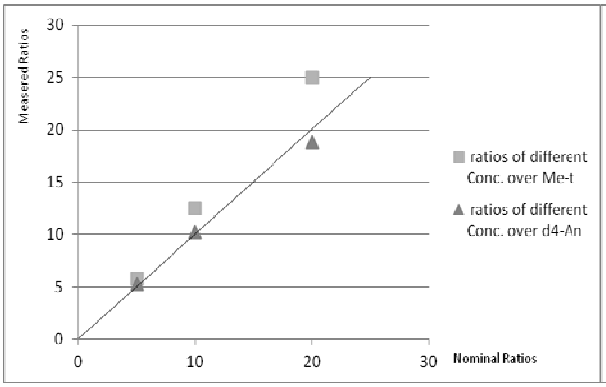

Fig7: quantification results of An with d_4-An and Me-t as internal standards (a series of different concentration of Etio existed)

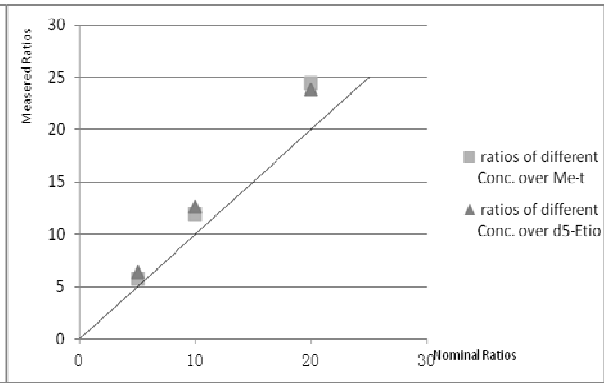

Fig8: quantification results of Etio with d_5- Etio and Me-t as internal standards (a series of different concentration of An existed)

Conclusion:

In routine practice, the concentrations of endogenous vary in a large range, not only between individuals but also between times of one person [4]. So our paper concludes it is always valuable to pay attention on the effect of internal standard on screening semi-quantitation results.

References:

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[4] P. Van Renterghema, P. Van Eenooa, H. Geyerb, W. Schänzerb, F.T. Delbekea. (2009) Alternative Steroid Profiling: Reference ranges for urinary endogenous steroids in a caucasian population of athletes. In: Schänzer W, Geyer H, Gotzmann A, Mareck U. (eds.) *Recent Advances in Doping Analysis (17)*, Köln, pp 81-90.