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Trilogy; Ionization procedures to detect corticosteroids abuse by means of LC/MS

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Three analytical procedures for urine corticosteroids screen have been compared. The sensitivity of HPLC did not fulfil the applicable standard known as Minimum Required Performance Limits (MRPL), and many of the target compounds were missed or detected as the degradation products by GC/MS screening. LC/MS was found to be the optimal platform, which could detect wide array of native form corticosteroids below the MRPL. Three types of liquid ionization procedures e.g., electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI) and atmospheric pressure photospray ionization (APPI) have been compared in this studies, and the results were discussed in this paper.

MATERIALS AND METHODS

Reference materials were purchased either from Sigma-Aldrich Japan K.K. (Tokyo, Japan) or from Steraloids Inc. (Newport, RI, U.S.A.). Diethylether was distilled from CaH_2 prior to use. Other reagents were of analytical grade. The Q-TOF MS instrument was a QSTAR XL MS/MS System from Applied Biosystems (Foster City, CA, U.S.A.) and the GC/MS was a HP5970MSD from Agilent Technologies (Hachioji, Tokyo, Japan)

Extraction

2.0 ml of urine specimen was placed in a test tube and 0.1 μ g of 19-nortestosterone was added as an internal standard. After addition of 0.1g of solid buffer Na_2CO_3 / $NaHCO_3$ = 2:1 and 0.5 g of sodium sulfate 10-H_2O, corticosteroids were extracted with 5ml of diethylether. The solvent was evaporated to dryness under a nitrogen stream and reconstituted with 50 μ l of 1%-acetic acid: acetonitryl (65:35). 20 μ l of the extract was fed on the LC or LC/MS. For GC/MS studies, an enol-TMS derivative[1)], a methoxyme-TMS derivative[2)] or a methyl cyclicboronate[3)] was formed, and 2 μ l of the derivative was injected onto GC column.

1) M.Donike, M.Ueki et.al: *J.Sports Med. Phys. Fitness*, **35**, p.1-99(1995)

2) M.Ueki et.al. in: *Recent Advances in Doping Analysis* (**3**) M.Donike, et.al. Ed., p.115 (Verlag Sport und Buch Strauß, Köln,1996)

3) Polettini A et.al.: *J Chromatogr*. **564**, p.529-535(1991)

Table-1 Analytical Conditions

HPLC Conditions			
Instruments:	HPLC1100(Agilent)		
Column:	ZORBAX Eclipse XDB-C8 4.6mm x 150mm; Column Temp.: 25^{o}		
Mobile phase	A: 1%-CH_3COOH	B: CH_3CN Flow rate: 0.25ml/min.	
Gradient:	0-3 min. B:35%	10min. B:60%	15min. B:65%
	16-21min. B:90%	25min. B:35%	
LC/MS Conditions			
Instrument:	QSTAR XL MS/MS System (Applied Biosystems)		
Acquisition mode:	TOF-MS	m/z: 140 to 600	
	ESI+	**APPI+**	**APCI+**
Ionspray Voltage:	5,500V	1,300V	-
Ionspray Temp.:	450^{o}C	400^{o}C	-
Nebulizer Gas:	Zero Air: 2.85 l/min	N_2: 3.94 l/min	Zero Air: 50 psi
Nebulizer Current:			2A
Auxially Gas:	Zero Air: 4.80 l/min	N_2: 2.00 l/min	Zero Air: 3.00 l/min
Dopant:	None	Toluene 20µl/min	None
Temp:	-	-	300^{o}C

RESULTS AND DISCUSSION

Most of the target compounds could be detected by HPLC with the limit of detection about 50 ng/ml, but the sensitivity did not reach the MRPL set by WADA. Even though the certain corticosteroids, such as Cortisone, Cortisol etc., could be detected with the acceptable sensitivity by GC/MS, many other target steroids were observed only as an artifact or multiple peaks (table-2). These results suggest that some of corticosteroids are decomposed during the GC run if not the chemical transformation can be successfully completed. Furthermore, oxosteroids, such as cortisone, were detected as syn- and anti-isomers after methoxime-TMS derivatization (figure-1). All three of the liquid ionization procedures allowed detecting any target compounds fairly below the MRPL. Sensitivities of APCI for the individual target steroids relative to ESI fell between 15 and 300% and seemed not the best for the comprehensive screening. APPI appeared to be the best sensitive ionization for cortisone(230%), cortisol(1,309%), corticosterone(352%), fludrocortisone(320%), fluticasone propionate(849%) and prednisone(517%), and seemed to be suitable for determining endogenous corticosteroids. APPI is known to be more effective for non-polar compounds than either APCI or ESI[4-5], and the ionization involves photoexcitation, photodissociation and recombination, namely, charge exchange or proton transfer[6]. Thus, sensitivity of APPI could be magnified by the addition of toluene as a dopant.

4) Yanxuan C et.al.: *Rapid Commun.Mass Spectrom.*, **19**, p.1717-1724(2005)

5) Leinonen A, et,al. *J Mass Spectrom,* **37,** p.693-698(2002)

6) Raffaelli A and Saba A : *Mass Spectrom Reviews,* **22**, p.318-331(2003)

The advantage of the use of APPI is that there does not seem to be any formation of cluster ions, resulting in an easier interpretation of analytical results and discovery of drug metabolites. Such distinctive feature of APPI seems to be particularly useful for pharmacokinetic studies and comprehensive screening of non-derivatized neutral steroids.

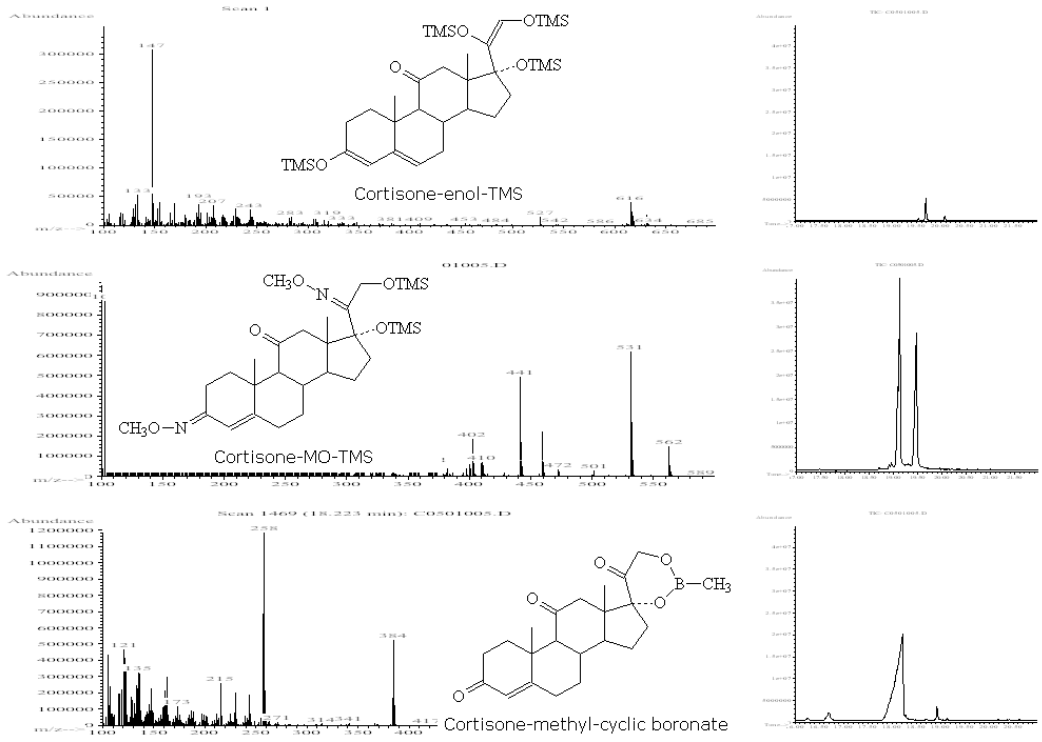

Figure-1 Detection of three cortisone derivatives by GC/MS

Table-2 Corticosteroid screen by conventional HPLC and GC/MS

Name	MRPL	HPLC/UV LOQ	S/N ratio ; GC/MS 1µg Injection		
			Enol-TMS	MO-TMS	Boronate
Triamcinolone			3	30	×
Fludrocortisone			3 peaks	20	40
Triamcinolone acetonide			2 peaks	2 peaks	20
Cortisone			120	100	100
Prednisolone			20	30	10
Betamethasone			10	artifacts	10
Cortisol			60	160	10
Corticosterone			100	30	×
Dexamethasone	30ng/ml	50ng/ml	20	artifacts	10
Prednisone			3 peaks	20	80
Beclomethasone			×	×	10
Fluorometholone			800	30	×
6a-Methylprednisolone			40	90	×
Medrysone			120	1400	×
Desonide			40	100	×
Flumethasone			90	artifacts	×
Budesonide			2 peaks	artifacts	×

Table-3 Signal to noise ratio at 10 ng of corticosteroids and relative sensitivity to ESI

	ESI	APCI		APPI	
	S/N	S/N	%	S/N	%
Triamcinolone	616	138	22.4%	270	43.9%
Fludrocortisone	295	248	84.1%	943	320.1%
19-nortestosterone	579	221	38.3%	1,232	212.9%
Triamcinolone acetonide	1,554	319	20.5%	2,205	141.9%
Cortisone	674	211	31.4%	1,549	229.9%
Prednisolone	536	113	21.1%	314	58.7%
Betamethasone	199	44	22.4%	134	67.3%
Cortisol	73	30	41.5%	952	1309.1%
Corticosterone	354	343	97.1%	1,243	351.5%
Dexamethasone	443	66	15.0%	234	52.9%
Prednisone	356	343	96.4%	1,841	517.3%
Fluticasone Propionate	1,489	4,748	318.8%	12,644	848.9%
Beclomethasone	430	104	24.1%	416	96.8%
Fluorometholone	653	612	93.8%	1,232	188.7%
Methylprednisolone	1,099	158	14.3%	472	43.0%
Medrysone	2,003	533	26.6%	2,173	108.5%
Desonide	2,044	797	39.0%	3,105	151.9%
Flumethasone	978	169	17.3%	1,203	123.0%
Budesonide	1,058	323	30.6%	1,874	177.2%

S/N ratio of signal at 10ng, resolution R=6000

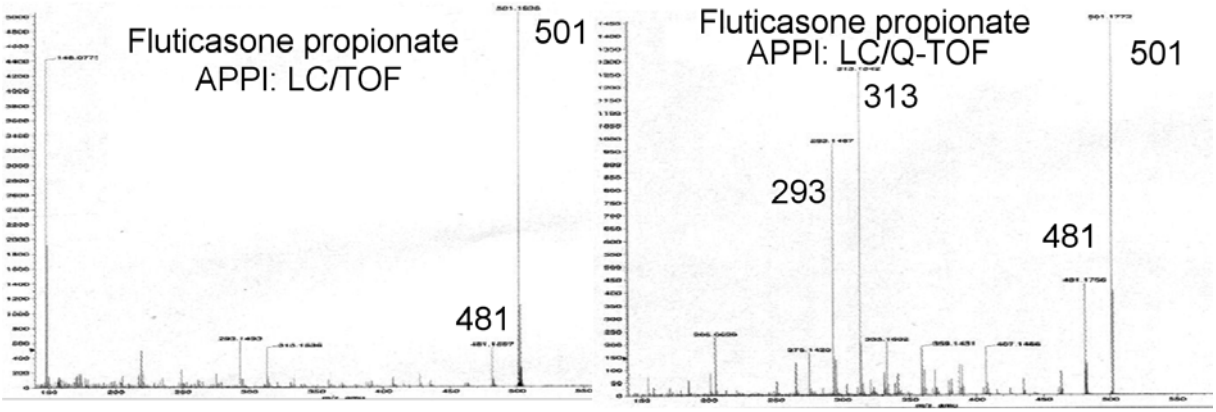

Figure-2 APPI-TOF and APPI-QTOF spectrums demonstrating absence of any cluster ion.

CONCLUSION

LC/ESI-MS was considered to be the suitable combination for the comprehensive screening of heat labile dope agents, and LC/APPI-MS was appeared to be the excellent ionization for measuring some endogenous and synthetic corticosteroids without any chemical transformation.