B. Starcevic, A. Butch

Use of LC/MS^3 on a new quadrupole linear trap mass spectrometer and high resolution chromatography to improve detection of anabolic steroids, glucocorticoids, anti-estrogenic agents, β_2-agonist and their metabolites

UCLA Olympic Analytical Laboratory, Los Angeles, USA

Abstract

The use of MS^3 mode to eliminate interferences often present in MS/MS mode in LC/MS/MS methods used to screen for anabolic steroids, glucocorticoids, anti-estrogenic agents, β_2-agonists and their metabolites, and the use of MS^3 mode as additional identification tool comparable to MS/MS spectra is presented.

Introduction

In the MS^3 mode a selected product ion is fragmented once more in the linear ion trap, resulting in the lower background and elimination of interferences as the chance of interference having the same two transitions as target compound is extremely small.
In order to improve current screening procedures for prohibited substances we are planning to develop a method that uses MRM as a basic scan type and then triggers a MS^3 to help eliminate background interference and improve compound identification.(1) We evaluated MS^3 mode parameters that would be beneficial for screening of anabolic steroids, glucocorticoids, anti-estrogenic agents and β_2-agonists.

Materials and Methods

Negative certified urine was spiked with compounds at MRPL levels or at concentrations present in the positive control urine or extracted calibrator. The concentrations are provided

in the table. Urine samples were extracted using our regular method consisting of hydrolysis with β-glucuronidase (*E.Coli)* followed by liquid-liquid extraction with a mixture of pentane and ethyl-ether. Dried extracts were reconstituted in 50 µL of mobile phase (2).
A QTrap 5500 Mass Spectrometer (ABSciex, Foster City, CA USA) was connected to an UFLC LC20-D binary pump model HPLC equipped with a SIL-20AC HT autosampler.
A Kinetex 2.6 µ C18, 50x2 mm column with a cartridge pre-column (Phenomenex, Torrance, CA USA) was used. A gradient elution method was used starting at 85% 5mM ammonium acetate buffer (adjusted to pH of 3.5) and 15% acetonitrile. Acetonitrile was increased to 95% in 7 minutes and kept constant for the next two minutes. Equilibration time was 3 minutes. The flow rate was 0.3mL/min and the injection volume was 5 µL. The mass spectrometer electrospray ionisation source was operated in positive mode for most compounds at a declustering potential of 5500V at 650^{o}C. .

Results and Discussion

Name	Conc. ng/ml	Precursor	Precursor 2	MS3	S/N	DP	CE	EE
Anabolic Steroids								
clenbuterol	10	277.2	203	131	300	120	23	0.10
epitrenbolone	10	271.1	199	166	93	120	73	0.11
hydroxymethylfermebolone	20	347.2	281	90-230	2000	85	21	0.10
gestrinone	40	309.2	241	157	3700	100	35	0.12
oxandrolone	20	307.2	271	253	52	120	22	0.10
tetrahydrogestrinone	30	313.2	241	157	323	70	33	0.10
methyltrienolone	50	285.2	107	76-81	102	82	34	0.10
methyldienolone	50	287.2	161	133	16	70	38	0.11
Glucocorticoids								
16α-OH-prednisolone	30	377.1	323	150-306	33	50	19	0.10
budesonide	30	431.3	323	259-288	2000	63	20	0.10
ciclesonide	30	541.2	393	260-324	400	66	24	0.10
deflazacort	30	442.2	225	207	195	55	43	0.11
des-acetyl-deflazacort	30	400.2	265	223	37	80	34	0.10
methylprednisolone	30	375.2	253	211	313	50	18	0.10
prednisolone	30	361.1	325	278-308	83	48	16	0.12
prednisone	30	359.2	313	213	1515	62	19	0.11

triamcinolone	30	395.2	375	175-358	1000	80	15	0.10
triamcinolone acetonide	30	435.2	415	355-396	400	82	16	0.10
des-iso-butyryl-ciclesonide	30	471.3	277	235	29	70	21	0.10
β-blocker								
carvedilol	10	407.3	224	80-220	3360	71	32	0.10
β_2–Agonists								
fenoterol	100	304.2	286	106-136	500	65	21	0.10
mepindolol	100	263.2	186	158	420	61	28	0.10
bambuterol	100	368.2	294	249	10000	67	27	0.10
terbutaline*	100	226.2	226	152	257	52	23	0.11
salmeterol	50	416.2	398	230-381	6000	80	40	0.10
Anti-estrogenic agents								
finasteride	50	373.2	305	185-191	19000	90	42	0.10
4-OH-tamoxifen	50	388.2	343	128-250	487	70	32	0.10
aminogluthetimide	50	233.2	146	131	> 20000	60	20	0.12
anastrazole	50	294.2	225	90-226	200	95	33	0.10
clomiphene	50	406.2	297	219	838	82	33	0.10
dutasteride	50	529.2	461	187	> 20000	70	51	0.10
examestane	50	297.2	121	90-94	160	85	32	0.10
fadrazole	50	224.2	156	129	153	90	49	0.10
fulvestrant	50	607.2	589	467	1081	40	27	0.10
3-OH-4-metoxytamoxifen	50	418.2	221	193	3600	68	36	0.11
N-desmethyltamoxifen	50	358.2	285	189-208	225	80	29	0.11
raloxifen	50	474.2	269	213	1124	100	44	0.10
testolactone	50	301.3	121	75-94	550	60	40	0.10

Table. 1 Compounds optimized and analyzed using MS^3 mode.

Majority of compounds tested showed excellent S/N ratios and in many cases the background was completely eliminated resulting in lowering the LOD for the compound.

For the compounds studied, the MS^3 mode was found to be a powerful tool for eliminating chromatographic interferences and can be used either for confirmation testing or in MRM screening for compound identification.

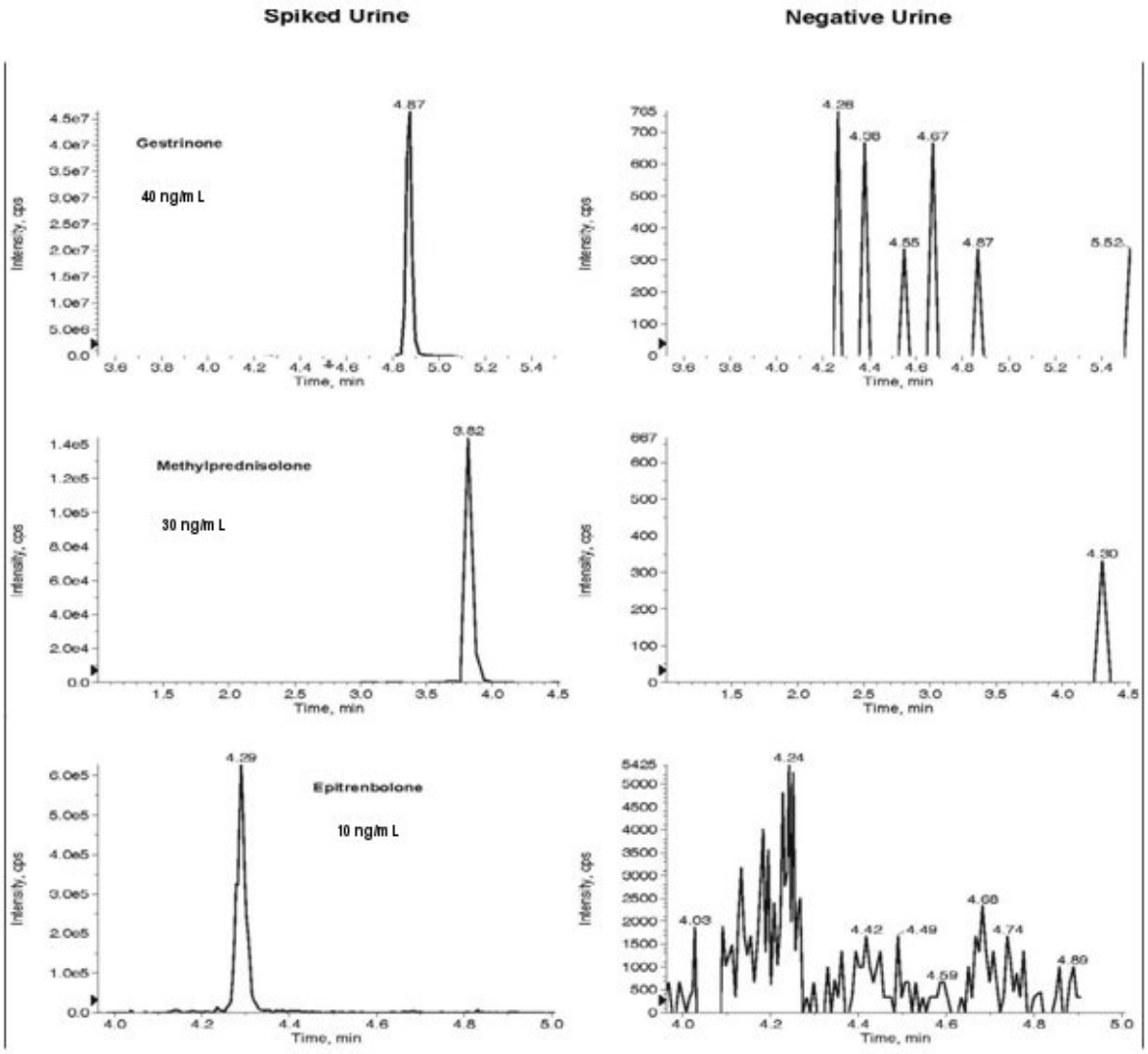

Figure 1. Examples of S/N ratios using MS^3 mode, note that the negative urine chromatograms are not at the same scale as spiked urine chromatograms.

References

1. Hopfgartner G, Varesio E, Tschappat V, Grivet C, Bourgogne E, Leuthold LA.(2004) Triple quadripole linear ion trap mass spectrometer for analysis of small molecules and macromolecules. *J. Mass Spectrom.* **39**,845-55.
2. Ahrens B, Starcevic B, Butch AW(In Press) Detection of prohibited Substances by Liquid Chromatography Tandem Mass Spectrometry for Sports Doping Control. In: Langman LJ, Snozek CLH in Drug Analysis: Methods and Protocols, New York, Springer Science + Business Media LLC.