Systematic screening for basic and acidic drugs in urine using Ultrafast-GCMSMS in MRM-scan mode – Part 1

Clayton Ross[1], Samantha Nelis[2], Mark Jarrett[2], Nigel Grieves[3], Paul Wynne[4]

[1]Shimadzu Australasia, Brisbane; [2]Queensland Government Racing Science Centre, Albion, Queensland; [3]Shimadzu Australasia, Rydalmere;
[4]Shimadzu Australasia, Melbourne, Australia (email: paul.wynne@shimadzu.com.au)

INTRODUCTION

The inherent power of Systematic Toxicological Analysis (STA) is increased when it is combined with non-targeted analysis and profiling of endogenous compounds. The use of profiles has been used for the detection of new or unexpected substances of concern, 'passporting' of individuals, etiological mapping across groups and to demonstrate physiological effect. When applied in biological systems, these techniques are broadly described as Vitaeomics. Our interests in STA are at odds with the current industry trend towards fully targeted multiple-analyte analysis. We view with interest the inclusion of non-targeted data to increase the informing power of our analyses, particularly in the area of performance-sports based residual-drug testing.

In this method, we favour GCMS for its orthogonality to LCMS, peak capacity, inlet selectivity and the informing power of full scan spectra. We also wish to exploit GCMSMS for the detection and possible quantitation of target compounds. We believe that the use of simultaneous scan for non-targeted screening and MRM for targeted analysis has the potential to improve workflow and also support profiling and unknown detection in the analysis of urine specimens.

EXPERIMENTAL

Urine samples were spiked with a variety of drug standards, β-glucuronidase hydrolysed and extracted using mixed mode SPE. The acidic fraction was methylated and the basic fraction was acetylated. Both were analysed using a GCMS-TQ-8030 (Shimadzu) with a Rxi-5Sil MS column (30 m x 0.25 mm, 0.25 µm, Restek). Injection of 1 µL of sample was splitless at 260ºC for 1 min. The temperature was held at 100ºC for 1.5 min and then ramped to 320ºC at 25ºC/min. The carrier gas was helium at a linear velocity of 37.2 cm/sec. The interface temperature was 280ºC and the source temperature was 220ºC. Each analyte was monitored by either a SIM or MRM event with a dwell time of 30 msec. The sample profile was monitored by a simultaneous scan event from 50-550 Da at a scan rate of 20000 Da/sec. For our own reporting convenience, we have divided the chromatogram into 7 time blocks.

substance	retention time	parent ion	quantifier	(CE)	qualifier	(CE)	qualifier	(CE)
Ibuprofen-d3 ME	6.173	164.2	122.1	5.0	120.0	20.0	93.0	25.0
Ibuprofen ME	6.18	161.0	117.0	10.0	119.0	25.0	91.0	20.0
Benzocaine	6.4	120.0	92.0	10.0	65.0	20.0		0.0
Amylobarbitone ME_2	6.525	184.0	169.0	10.0	112.0	18.0	83.0	25.0
Pentobarbitone ME_2	6.68	184.0	169.0	10.0	112.0	20.0	83.0	25.0
Phenacetin	6.94	179.0	137.0	8.0	109.0	20.0		0.0
Phenazine	7.25	180.0	152.0	20.0		0.0		0.0
Caffeine	7.61	194.0	109.0	15.0	55.0	18.0		0.0
Phenobarbitone ME_2	7.66	232.0	175.0	10.0	118.0	20.0	90.0	25.0
Diflunisal	7.65	204.0	175.0	15.0	156.0	10.0		0.0
Flufenamic acid ME	7.7	263.0	140.0	5.0	166.0	10.0	194.0	25.0
Fenoprofen ME	7.79	197.0	104.0	15.0	91.0	15.0	78.0	30.0
Flurbiprofen ME	7.86	199.0	178.0	25.0	199.0	10.0		0.0
Niflumic acid ME	8	295.0	263.0	20.0	235.0	30.0		0.0
Diflunisal ME_2	8.1	247.0	204.0	20.0	169.0	5.0		20.0
Naproxen ME	8.22	185.0	153.0	18.0	170.0	10.0	141.0	30.0
Flunixin ME	8.26	295.0	263.0	15.0	235.0	30.0	208.0	30.0
Mefenamic Acid ME	8.56	223.0	208.0	10.0	180.0	20.0	152.0	35.0
Ketoprofen ME	8.65	209.0	105.0	12.0	77.0	30.0	165.0	30.0
Tolfenamic Acid ME	8.842	208.0	180.0	15.0	152.0	30.0	77.0	30.0
Ethacrynic Acid ME	8.826	243.0	208.0	20.0	179.0	35.0		0.0
Diclofenac ME	9.05	214.0	179.0	20.0	151.0	32.0	177.0	30.0
Phenytion ME	9.08	203.0	118.0	18.0	77.0	30.0		0.0
Etodolac ME	9.113	272.0	198.0	12.0	57.0	18.0	181.0	20.0
Eltenac ME	9.135	220.0	185.0	15.0	140.0	20.0		0.0
Suprofen ME	9.119	215.0	111.0	18.0	187.0	5.0		0.0
Tiaprofenic Acid ME_2	9.15	229.0	105.0	18.0	77.0	30.0		0.0
Tolmetin ME	9.285	212.0	120.0	10.0	119.0	20.0	91.0	30.0
Meclofenamic Acid ME	9.36	242.0	179.0	25.0	214.0	20.0	151.0	35.0
Phenylbutazone	9.38	183.0	77.0	15.0	51.0	30.0		0.0
Bufexamac ME_2	9.35	163.0	107.0	10.0	77.0	20.0		0.0
Ketorolac ME	9.53	210.0	105.0	5.0	131.7	20.0	104.5	35.0
Zomepirac ME	9.62	246.0	139.0	20.0	134.0	10.0	111.3	30.0
Vedaprofen ME	9.82	237.0	155.0	15.0	127.0	35.0	153.0	35.0
Pentoxifylline	9.98	221.0	193.0	15.0	150.0	12.0	109.0	25.0
Diazepam	10.16	256.0	221.0	15.0	165.0	32.0	206.0	32.0
Carprofen ME_2	10.75	242.0	206.0	5.0	191.0	35.0		25.0
Temazepam M e	10.784	271.0	193.0	15.0	165.0	20.0	178.0	25.0
Temazepam	10.847	271.0	193.0	15.0	165.0	20.0	178.0	25.0
Clanobutin ME	11	139.0	111.0	15.0	75.0	28.0		0.0
Carprofen ME	11.02	228.0	193.0	18.0		0.0		0.0
Indoprofen	11.38	236.0	218.0	15.0	91.0	20.0	77.0	35.0
Furosemide ME_3	11.9	81.0	53.0	10.0	81.0	15.0	96.0	15.0
Indomethacin	12.12	139.0	111.0	15.0	75.0	30.0		0.0
Bumethanide ME_3	12.55	363.0	254.0	5.0	318.0	10.0	194.0	20.0
Sulindac ME	14.65	233.0	231.0	30.0	213.0	35.0		45.0
Heptaminol-Ac_2	5.5 to 6.15	114	56.0	20.0	44.0	20.0	113	5.0
Nikethamide	6.15 to 6.5	177	131.0	20.0	149.0	18.0	120	22.0
Phenazine	6.5 to 7.2	180	85.0	10.0				
Methylamphetamine Ac	6.5 to 7.2	100	100.0	5.0	58.0	10.0		
Methoxyphenylamine Ac	7.2 to 7.8	148	119.0	12.0	133.0	15.0	105	20.0
Antipyrine	7.8 to 8.15	188	96.0	10.0	105.0	12.0	84	10.0
MDMA Ac	8.15 to 8.4	162	104.0	18.0	131.0	20.0	103	20.0
Chlopheniramine	8.4 to 8.85	203	167.0	30.0	168.0	15.0	202	8.0
Tramadol Ac	8.4 to 8.85	188	173.0	8.0	160.0	8.0	134	8.0
Dextromethorphan	8.85 to 9.1	271	271.0	5.0				
Mepyramine	9.1 to 9.5	121	77.0	15.0	91.0	15.0	106	15.0
Levorphanol Ac	9.5 to 9.9	299	150.0	20.0	59.0	15.0	200	15.0
Pentazocine Ac	9.5 to 9.9	259	110.0	12.0	45.0	12.0	244	12.0
Benzydamine	9.9 to 10.2	85	70.0	10.0	58.0	10.0	85	3.0
Hydrocodone	10.2 to 10.45	299	242.0	20.0	228.0	20.0	198	15.0
D6 Codeine Ac	10.45 to 10.9	347	246.0	10.0		0.0		
Ethlymorphine Ac	10.45 to 10.9	355	204.0	15.0	296.0	10.0	162	30.0
Propoxycaine Ac	10.9 to 11.45	86	86.0	5.0				
RACP Ac	10.9 to 11.45	370	86.0	15.0	285.0	10.0	58	25.0
Nafronyl	10.9 to 11.45	86	99.0	10.0	86.0	3.0		
Azaperol Ac	10.9 to 11.45	107	78.0	15.0	51.0	30.0	80	10.0
Butorphanol Ac	11.45 to 11.8	314	284.0	10.0	296.0	10.0	131	35.0
Nalbuphine Ac_3	11.8 to 13.8	428	428.0	5.0				
Nalbuphine Ac_2	11.8 to 13.8	386	296.0	15.0	254.0	25.0	386	5.0
Diprenorphine Ac	13.8 to 18.5	428	428.0	5.0				

Table 1: Selected analytes included in the study with retention times (acquisition window for bases) and EI-MSMS parameters (precursor and product ions with applied collision energy). Ac is acetyl and Me is methyl derivative.

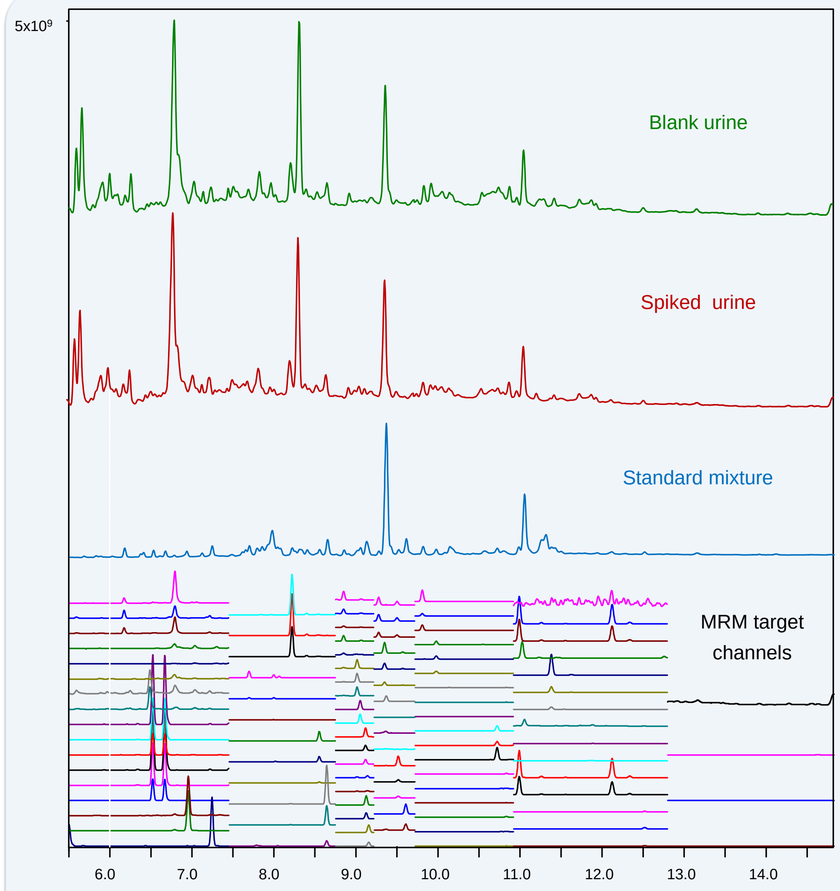
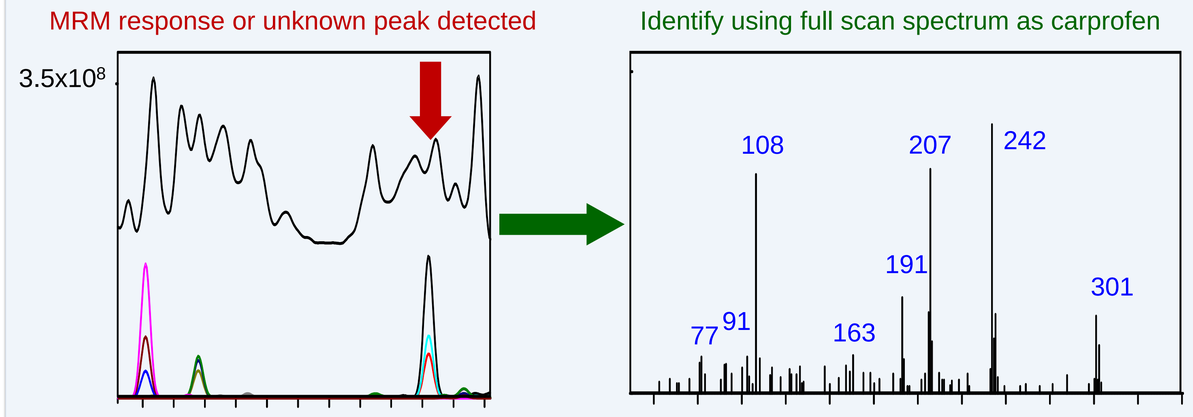


Figure 1: Full scan chromatograms for methylated acid extract of urine showing blank, spike and mixed drug standard and MRM channels for target screening. (Note some substances are not included in the spike).

Figure 2 (below): A segment of the chromatogram showing the full scan and MRM channels. Full scan data is available for carprofen (dimethyl) with a molecular ion at m/z 301 and a chlorine isotopic pattern. Matching the full scan spectrum confirms the more sensitive MRM response.



DISCUSSION

Using a scanning speed of 20000 Da/sec in a GCMSMS system with high sensitivity, we have demonstrated a method for the analysis of urine extracts using simultaneous scan and targeted MRM for both acidic and basic drugs residues (Table 1).

The choice of electron impact mode (EI-GCMSMS) is counterintuitive for high sensitivity MRM methods where it is desirable to concentrate the precursor ion into a single abundant species. In such cases, confirmation of identity is possible using a Product Ion Scan in combination with the MRM channel (data not shown). However, we consider that this approach is limiting in the detection and library based identification of non-target compounds and found that, in most cases, EI offers not only equally or more abundant ion populations than CI but also the choice of more than one high value precursor ion. The ability to chose precursor ions from the EI spectrum provides a facile route to reliable MRM products. In a few cases where EI fragmentation was extensive or yielded a single abundant low-mass fragment, we optionally included a SIM event for that fragment for sensitive detection in preference to MRM. (See Figures 1 and 2).

Of particular value to the method was the simultaneous background scan event. The data from this channel provided unequivocal identification of known compounds (responses in MRM channels) or distinctive spectra for the identification of unexpected compounds (demonstrated for carprofen in Figure 2). Of particular interest to us is the ability to use the EI fragmentation data and chromatographic elution patterns to profile endogenous compounds in equine and canine urine that allow us to detect changes in feeding and management practices. Additionally, the scan event provides a facile method for working with the unique metabolism of the horse, known in many cases to yield urinary metabolites that are either unknown in other species or of far greater importance than in other mammals. Such a capability gives us greater confidence in the detection of new substances that have not previously been investigated in the sport.

CONCLUSION

We report a GC-EI-MSMS method using MRMs for the detection of targeted compounds, the unequivocal confirmation of structure using EI-MS and a background full scan that is useful in detecting the presence of unexpected substances and changes to the normal sample matrix. The method is enabled by the use of a UFMS capable GCMSMS system that is capable of supporting simultaneous MRM-scan-PIS.

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Please contact the corresponding author for details of the proposed publication schedule for further parts of this study.