ELIMINATION PROFILES OF 6-METHOXY-2-NAPHTHYLACETIC ACID, THE MAJOR ACTIVE METABOLITE OF NABUMETONE IN HORSE SERUM AND URINE

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ABSTRACT

Nabumetone was administered orally as a single dose of 5 g to each of 4 horses. Urine and serum samples were collected at regular intervals up to 120 h post administration. The elimination profiles of 6-methoxy-2-naphthylacetic acid (6-MNA), the major active metabolite of nabumetone in man, in serum and urine samples of the 4 horses were generated based on quantification of the drug by a validated HPLC/UV method. The presence of 6-MNA in the samples was confirmed by GC/MS.

The maximum concentrations of 6-MNA in the urine samples of the 4 horses ranged from 74.4–172.4 µg/ml from 3–16 h after drug administration. The latest detection in urine was 54 h for 3 horses and 78 h for one horse. Peak serum concentrations were found at 3 h for one horse, 6 h for 2 horses and 12 h for one horse and ranged from 13.4–28.8 µg/ml. The latest detection in serum varied between 78 h and 102 h post administration.

INTRODUCTION

Nabumetone, (4-(6-methoxy-2-naphthalenyl)-2-butanone, is a non-steroidal anti-inflammatory drug (NSAID) sold under the trade name of Relafen (SmithKline Beecham Pharmaceuticals, West Sussex, UK). Its use is indicated for the relief of symptoms of osteoarthritis and rheumatoid arthritis (Boyle *et al.* 1982). Nabumetone is non-acidic and does not dissociate in the gastrointestinal tract after oral administration; as a result, it does not inhibit prostaglandin synthesis. However, it is a prodrug that undergoes hepatic biotransformation to the active metabolite, 6-methoxy-2-naphthylacetic acid (6-MNA) that is a potent inhibitor of prostaglandin synthesis The chemical structures of nabumetone and 6-MNA are shown in Figure 1. Following oral administration to people, it is well absorbed from the gastrointestinal tract with approximately 80% of the dose eliminated in the urine. It undergoes rapid biotransformation with approximately 35% of the dose converted to 6-MNA and 50% to other metabolites. Nabumetone is as effective as other NSAIDs with fewer side effects including a reduced tendency to induce gastric erosions (Soma *et al.* 1996).

As for other NSAIDs, HPLC is a suitable method of analysis. Other analytical techniques that have been applied include gas chromatography, gas chromatography/mass spectrometry, capillary electrophoresis/mass spectrometry and liquid chromatography/mass spectrometry (Dusci *et al.* 1979; De Kanel *et al.* 1998). In 1996, Soma *et al.* reported on a HPLC method for the detection of 6-MNA in equine plasma and urine. They determined that, as reported in other species, nabumetone is metabolised to 6-MNA in the horse. Following a 2.0 g oral dose of nabumetone, 6-MNA was still quantifiable in urine at 72 h post administration. They recommended that the drug should be withdrawn 96–120 h before racing.

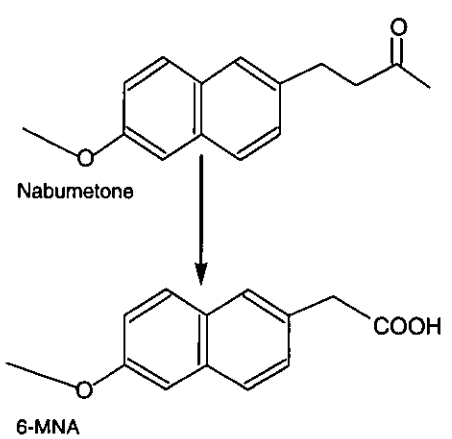

Fig 1: The chemical structure of nabumetone and its metabolite, 6-MNA.

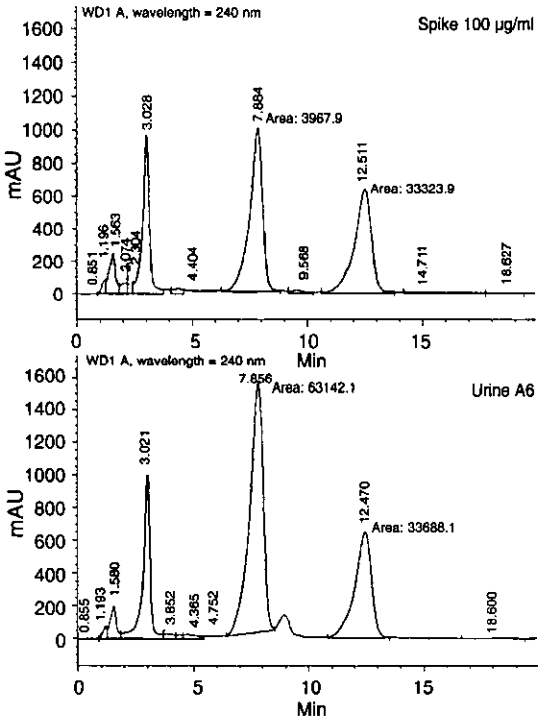

Fig 2: HPLC/UV chromatograms of an extract of a urine sample supplemented with 6-MNA and a 8-10 h (A6) post administration urine sample.

NSAIDs are frequently used in horses to reduce pain and inflammation associated with racing. However, they are generally prohibited by the rules of racing and are not permitted to be used on race day. Therefore, the purpose of the present study was to determine the elimination profile of 6-MNA in serum and urine samples after oral administration of a single dose of 5 g nabumetone to horses.

Materials and methods

The reference standard of 6-MNA was a gift from SmithKline Beecham Pharmaceuticals and naproxen was obtained from Syntex Ltd, USA.

Drug administration

Four Standardbred mares were administered a single oral dose of 5 g nabumetone as the product Relafen. Blood (18 samples) and urine (19 samples) were collected at regular intervals up to 120 h following drug administration. Blood samples were centrifuged immediately after collection and the serum was isolated. Urine and serum samples were frozen until analysis.

Urine extraction

Urine (1 ml) was supplemented with 50 µg/ml naproxen as the internal standard and conjugates were hydrolysed by adjusting the pH to 14 with 4 M sodium hydroxide and heating in a 70°C water bath for 30 min. After cooling to room temperature, the sample was adjusted to pH 2 with hydrochloric acid and extracted with 2 x 4 ml aliquots of petroleum ether:dichloromethane (60:10). The organic layers were combined and evaporated to dryness under nitrogen at 60°C. The residue was dissolved in 200 µl acetonitrile. For each of the 4 horses, pre-administration urine samples were supplemented in duplicate with 6-MNA at concentrations of 0.5, 1.0, 10, 100, 150 and 200 µg/ml for the construction of the calibration curves. The QC samples at 10 and 50 µg/ml (in duplicate) were concurrently extracted and analysed.

Serum extraction

A volume of 2 ml serum was supplemented with 10 µg/ml naproxen as internal standard and the pH was adjusted to 2 using hydrochloric acid. The extraction was then carried out with 2 x 4 ml petroleum ether:DCM (60:10). The organic layers were separated, combined and dried under nitrogen at 60°C. The residue was dissolved in 200 µl acetonitrile. For each of the 4 horses, a calibration curve was constructed from the analysis of 6-MNA calibrators at concentrations of 0.1, 0.5, 1.0, 10 and 100 µg/ml in duplicate. The QC samples at 1.0 and 10 µg/ml (in duplicate) were concurrently extracted and analysed.

HPLC analysis

The extracts were analysed by HPLC under the following conditions:

Instrument:	HP 1100 Series HPLC
Column:	LiChroCART RP-18 (125 x 4 mm, 5 µm)
Mobile phase:	Acetonitrile:0.5 % aqueous acetic acid (35:65)
Flow rate:	1.0 ml/min
Detection:	UV @ 240 nm
Injection volume:	20 µl

GC/MS analysis

The presence of 6-MNA was confirmed using the conditions listed below following derivatisation of the extract with bis(trimethylsilyl)trifluoroacetamide (BSTFA).

Instrument:	HP 5890 GC; HP 5972 MSD
Column:	DB-1 capillary column, 30 m x 0.25 mm, 0.25 µm
Injector temperature:	280°C

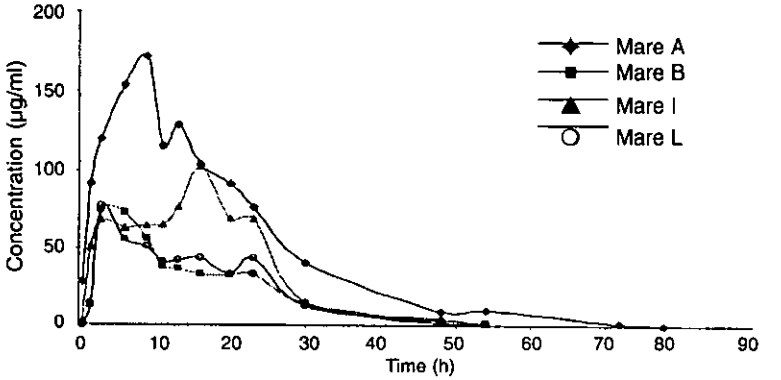

Fig 3: The elimination profile of 6-MNA in the urine of 4 horses after oral administration of 5 g nabumetone.

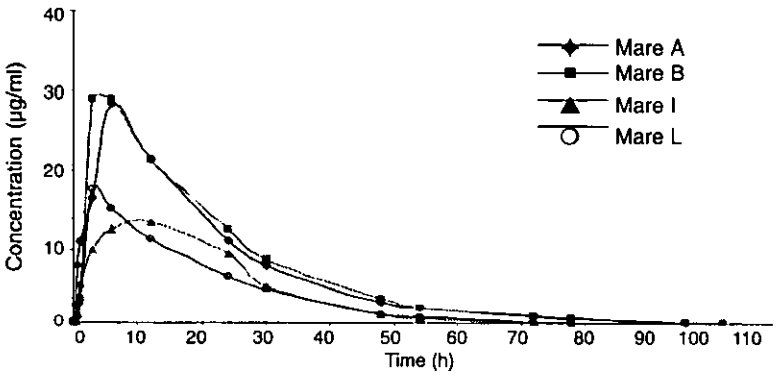

Fig 4: The elimination profile of 6-MNA in the serum of 4 horses after oral administration of 5 g nabumetone.

Detector temperature: 290°C
Initial oven temperature: 80°C
Initial hold time: 1 min
Temperature programme: 20°C/min.
Final temperature: 280°C
Injection volume: 1 µl

METHOD VALIDATION

The HPLC/UV method used for the determination of 6-MNA in urine and serum samples was validated prior to sample analysis. A plot of concentration versus peak area ratio resulted in a linear curve up to the highest concentration analysed (200 µg/ml for urine and 100 µg/ml for serum). The limit of detection (LOD) and limit of quantitation (LOQ) in urine were determined by analysing a set of 8 replicate urine samples supplemented with 6-MNA at 0.5 µg/ml (the estimated LOD) and 5 µg/ml of internal standard and in serum by analysing 8 replicate serum samples supplemented with 6-MNA at 0.1 µg/ml (the estimated LOD) and 1 µg/ml of internal standard. The ratios of the peak areas of 6-MNA to the internal standard were determined. The LOD and LOQ for the samples were calculated based on 'signal in blank matrix converted to concentration + 3 (standard deviation of the peak area ratios)' and

TABLE 1: Method validation data

	Urine			Serum		
Linearity	Up to 200 µg/ml			Up to 100µg/ml		
Recovery	99%			67%		
LOD	0.15 µg/ml			3.5 ng/ml		
LOQ	0.18 µg/ml			4.0 ng/ml		
Precision	**Concentration**	**Intra-day**	**Inter-day**	**Concentration**	**Intra-day**	**Inter-day**
	10 µg/ml	1.2%	0.9%	1.0 µg/ml	2.2%	2.0%
	50 µg/ml	1.5%	0.5%	10 µg/ml	0.5%	0.6%
	100 µg/ml	0.5%	0.2%	50 µg/ml	1.8%	1.2%
Accuracy	**Concentration**	**Value**		**Concentration**	**Value**	
	10 µg/ml	98.5% (cv 2.4%)		5 µg/ml	105% (cv 0.1%)	
	50 µg/ml	105% (cv 2.6%)		25 µg/ml	102% (cv 1.6%)	

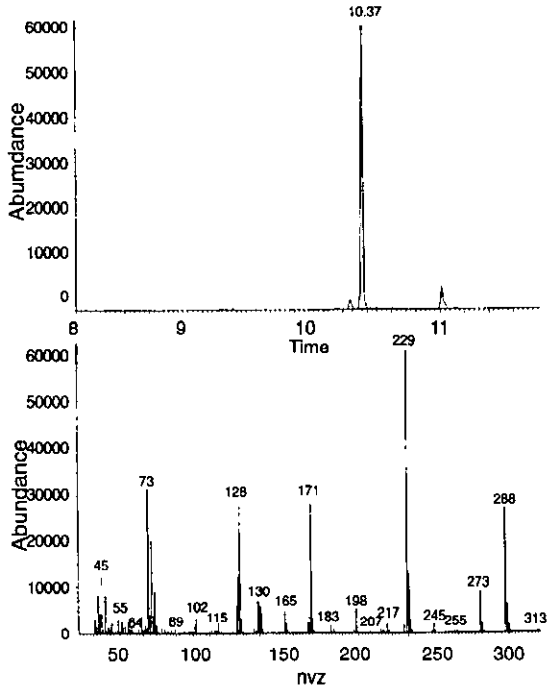

Fig 5: Extracted ion GC/MS chromatogram and the corresponding mass spectrum obtained for a TMS derivative of an extract of a urine sample collected 30 h post administration.

'signal in blank matrix converted to concentration + 10 (standard deviation of the peak area ratios)', respectively.

The precision of the method was evaluated by extracting and analysing 7 supplemented replicates on 3 consecutive days. Blank urine samples were supplemented with 6-MNA at 10, 50 and 100 µg/ml and blank serum samples were supplemented with 6-MNA at 1.0, 10 and 50 µg/ml. Accuracy was determined with sets of urine and serum samples supplemented by a different person (QC Officer). The samples for the calibration curve were prepared by the regular analyst. The accuracy was measured by analysing QC urine samples supplemented with 6-MNA at 10 and 50 µg/ml and serum samples supplemented with 6-MNA at 5 and 25 µg/ml in duplicate. The concentrations of 6-MNA in the QC samples were determined using the calibration curves.

The results of the method validation are presented in Table 1.

A drug stability study was carried out at 2 concentrations in both urine and serum: 10 and 50 µg/ml for urine and 1.0 and 10 µg/ml for serum. Aliquots (1 ml) of these samples were stored in duplicate under the following conditions:

- At room temperature (21–23°C) for 1, 2, 3 and 7 days.

TABLE 2: The 6-MNA concentrations in urine samples of 4 horses after oral administration of 5 g nabumetone

Time (h)	Concentration (µg/ml)			
	Horse A	Horse B	Horse I	Horse L
Pre	-	-	-	-
0–1	28.5	ND	2.69	ND
1–2	92.1	12.5	51.8	14.1
2–4	120	74.4	68.9	77.6
4–8	154	72.8	63.5	55.8
8–10	172	56.8	64.8	51.3
10–12	116	38.8	66.0	41.7
12–14	129	37.1	76.5	42.4
14–18	104	33.8	103	44.1
18–22	91.5	33.0	69.2	33.9
22–24	76.6	33.9	69.4	44.1
29–31	41.4	14.2	14.6	12.7
47–49	8.81	2.57	3.99	1.72
53–55	9.60	1.19	1.21	0.64
71–73	1.51	ND	ND	ND
77–79	0.30	ND	ND	ND
95–97	ND	ND	ND	ND
101–103	ND	ND	ND	ND
119–121	ND	ND	ND	ND

ND: Not detected

- At refrigerator temperature (3–4°C) for 1, 2, 3, 7 and 30 days.
- At freezer temperature (-18°C) for 1, 2, 3, 7, 30 and 45 days.
- Supplemented urine subjected to 1, 2, 3 and 5 freeze and thaw cycles.

No degradation was observed under these conditions.

Results

The calibration curves used for the quantification of 6-MNA in urine and serum had coefficients of determination (R^2) of 0.994 or better.

Concentrations of 6-MNA in the urine samples of 4 horses are listed in Table 2. The highest 6-MNA concentrations were 172 µg/ml (Horse A), 74.4 µg/ml (Horse B), 103 µg/ml (Horse I) and 77.6 µg/ml (Horse L) at 8–10 h for Horse A, 2–4 h for Horses B and L and 14–18 h for Horse I. 6-MNA was detected from the first hour in Horses A and I and the last detection was 78 h for Horse A and 54 h for Horse I. For Horses B and L, the drug was detected in the 1–2 h collection and was last detected in the sample collected at 53–55 h post administration.

The concentrations of 6-MNA in serum samples of 4 horses are listed in Table 3. The peak concentration was 28.3 µg/ml (6 h, Horse A), 28.8 µg/ml (6 h, Horse B), 13.4 µg/ml (12 h, Horse I) and 17.5 µg/ml (3 h, Horse L). 6-MNA was detected up to 96 h in the serum of Horse A, 102 h in Horse

TABLE 3: The 6-MNA concentrations in serum samples of 4 horses after oral administration of 5 g nabumetone

Time (h)	Concentration (µg/ml)			
(h)	Horse A	Horse B	Horse I	Horse L
Pre	-	-	-	-
5 min	ND	ND	ND	ND
10 min	0.20	0.11	ND	ND
20 min	2.74	0.30	0.08	ND
40 min	7.96	0.86	1.21	1.25
1 h	11.0	3.07	5.42	3.78
3 h	16.5	28.8	9.88	17.5
6 h	28.3	28.8	12.5	15.1
12 h	21.4	21.3	13.4	11.3
24 h	11.2	12.5	9.41	6.55
30 h	8.01	8.73	5.29	4.81
48 h	3.08	3.71	1.16	1.37
54 h	2.22	2.34	0.63	0.90
72 h	0.88	1.01	0.10	0.20
78 h	0.58	0.75	0.02	0.14
96 h	0.02	0.14	ND	ND
102 h	ND	0.08	ND	ND
120 h	ND	ND	ND	ND

ND: Not detected

B and 78 h in Horses I and L. Representative HPLC/UV chromatograms are presented in Figure 2. Figures 3 and 4 show the elimination profiles in urine and serum, respectively.

The identity of the peak detected by HPLC was confirmed as 6-MNA by GC/MS after derivatisation with BSTFA. Figure 5 presents the extracted ion chromatogram and mass spectrum obtained from the analysis of a urine extract.

Conclusions

A HPLC/UV method was validated for the detection of 6-MNA in equine urine and serum. Samples collected after an oral administration of 5 g nabumetone were analysed and the elimination profiles determined. Oral administration of 5 g nabumetone can be detected for 102 h post administration using the validated methods reported in this paper.

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