# DETECTION AND DISPOSITION OF PYRILAMINE AND ITS METABOLITE O-DESMETHYLPYRILAMINE IN EQUINE BLOOD AND URINE: A PRELIMINARY REPORT

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## ABSTRACT

Pyrilamine is an antihistamine used in human and equine medicine. Because antihistamines produce central nervous system effects in horses, pyrilamine is classified as an ARCI Class 3 substance, which has the potential to affect the performance of racehorses. The predominant equine urinary metabolite is O-desmethylpyrilamine (O-DMP). The ELISA and GC/MS detection of pyrilamine and/or O-DMP in equine blood and urine samples, following oral and iv administration, are reported.

Pyrilamine and O-DMP are detected readily in equine blood and urine by the pyrilamine ELISA and GC/MS selected ion monitoring (SIM) methods used. After iv injection of pyrilamine (100 mg), the ELISA detected pyrilamine equivalents in serum for up to 11 h and in urine for up to 69 h. We used the GC/MS-SIM method to quantify pyrilamine and O-DMP in equine blood and urine. After iv administration of pyrilamine (300 mg/horse), serum pyrilamine concentrations declined from about 350 ng/ml at 5 min post dose to about 6 ng/ml at 8 h post dose. After oral administration of pyrilamine (300 mg/horse) serum concentrations reached about 20 ng/ml at 1 h post dose, falling to about 0.6 ng/ml at 8 h post dose. No pyrilamine was detected in serum at 24 h post dose by either route.

Recovery of O-DMP from urine was optimised by incubating the urine samples for about 12 h at 37°C with *Helix pomatia* ß-glucuronidase. Hydrolysis was followed by solid phase extraction (SPE), trimethylsilyl derivatisation and GC/MS-SIM analysis to determine the apparent O-DMP concentrations. After iv injection of pyrilamine (300 mg/horse) O-DMP was recovered at a level of about 40 µg/ml at 1 h post dose thereafter declining to about 4 ng/ml at 168 h post dose. After oral administration, the O-DMP recovery peaked at about 28 µg/ml at 6 h post dose and declined to about 3 ng/ml at 168 h post dose.

Preliminary pharmacokinetic analysis of serum pyrilamine concentrations following iv and oral administration suggests relatively low (12%) bioavailability of pyrilamine in horses. However, the apparent urine concentrations of O-DMP after iv and oral administrations were essentially equivalent at 6 h post dose, suggesting rapid oral absorption and extensive first-pass metabolism followed by enterohepatic circulation of this agent.

## INTRODUCTION

Pyrilamine (N-[(4-methoxyphenyl)methyl]-N,N-dimethyl-N-2-pyridinyl-1,2-ethanediamine) is an ethylenediamine type antihistamine that is an $H_1$-receptor antagonist used extensively in human and veterinary medicine for symptomatic relief of allergic reactions. Although the behavioural effects of pyrilamine on the horse are unknown, general side effects of antihistamines include sedation or central nervous system stimulation, depending on the medication, dose and route of administration (Douglas 1985). Pyrilamine is classified by the Association of Racing Commissioners International (ARCI) as a Class 3 agent, and its use in racehorses is prohibited because it may alter their performance. Therefore, methods for detection and identification of antihistamines and/or their metabolites in biological samples from horses are needed for effective control of these agents. Detection of pyrilamine and/or its metabolites in post race samples may lead to significant sanctions against trainers.

Detection and identification of pyrilamine in urine samples has been hampered because the drug is metabolised extensively, and only small amounts of the parent drug are excreted in urine. The need for studies is accentuated by the fact that

the immunoassay tests for pyrilamine can be highly sensitive to the urinary metabolites of pyrilamine which appear to remain detectable for long periods after administration of this agent. Recent research has indicated that the predominant equine urinary metabolite is O-desmethylpyrilamine (O-DMP), which is formed by the removal of a methyl group (Harkins *et al.* 2000). The objectives of this study were to develop analytical methods for the detection of pyrilamine and its metabolite in biological fluids after iv and oral administrations and to examine the relationships between dose, route of administration, pharmacological response and analytical findings.

## MATERIALS AND METHODS

### *Horses*

Mature Thoroughbred mares weighing 485–576 kg were used. The animals were maintained on grass hay and feed (12% protein) which was a 50:50 mixture of oats and an alfalfa-based protein pellet. Horses were fed twice daily. The mares were vaccinated annually for tetanus and were de-wormed quarterly with ivermectin (MSD Agvet, New Jersey, USA). A routine clinical examination was performed before each experiment to ensure that the animals were healthy and sound. During experimentation, horses were provided with water and hay *ad libitum*. All animals used in the experiments were managed according to the rules and regulations of the Institutional Animal Care Use Committee at the University of Kentucky, which also approved the experimental protocol.

### *Pyrilamine administration*

Horses were given pyrilamine (Sigma Chemical Co, Missouri, USA) according to one of the following protocols: 100 mg iv, 300 mg iv or 300 mg orally. From horses receiving the 100 mg doses, urine was collected at 0, 2, 4, 6, 11, 24, 48 and 69 h post dose and serum was collected at 0, 0.5, 1, 2, 4, 6, 11, 24, 48 and 69 h post dose. From horses receiving the 300 mg doses, urine was collected at 0, 1, 2, 4, 6, 8, 24, 48, 72, 96 and 168 h and serum at 0, 5, 10, 15, 30 min and 1, 2, 4, 8, 24 and 48 h post dose.

### *Reagents*

Pyrilamine maleate, tripelennamine HCl, pheniramine maleate, chlorpheniramine maleate, diphenhydramine HCl and albuterol were obtained from Sigma Chemicals, Missouri, USA. O-DMP was prepared as described by Harkins *et al.* (2000). N,O-bis(trimethylsilyl)trifluoroacetimide with 1% trimethylchlorosilane (BSTFA with 1% TMCS) was obtained from Pierce, Illinois, USA.

### *ELISA cross-reactivity*

Standards for ELISA cross-reactivity studies were prepared in methanol and diluted to appropriate concentrations in assay buffer. The pyrilamine ELISA (Neogen, Kentucky, USA) was performed as described by Rees *et al.* (1996). Briefly, the assays were started by adding 20 µl of the standard to each antibody coated well, along with 180 µl of a pyrilamine-horseradish peroxidase (pyrilamine-HRP) solution. The wells were allowed to incubate for 1 h, followed by washing and the addition of 150 µl K-Blue Substrate (Neogen, Kentucky, USA). The optical density (OD) at a wavelength of 650 nm was measured with an automated microplate reader (Bio-Tek Instruments, Vermont, USA) approximately 30 min after addition of the substrate. All assay reactions were run at room temperature.

### *ELISA quantification of pyrilamine equivalents*

The urine and serum samples were screened with the pyrilamine ELISA to determine the concentrations of pyrilamine equivalents (ie the concentration of pyrilamine that would produce similar results) in the samples. A standard curve of pyrilamine in urine or serum was used to quantify the samples. The assays were started by adding 20 µl of the standard, test, or control samples, to each well, together with 180 µl pyrilamine-HRP solution to wells containing the test samples. A quantity of 160 µl of the pyrilamine-HRP solution was added to wells containing standard and control samples along with 20 µl of blank urine/serum to create a matrix comparable to the test samples. Thereafter, the ELISA was performed as above.

### *O-desmethylpyrilamine-glucuronide analysis by MS/MS*

Direct infusion injection of urine samples on a Micromass (Massachusetts, USA) Quattro II tandem mass spectrometer (MS/MS) was performed as described by Korfmacher *et al.* (1988) and Bosken *et al.* (2000). Full scan positive electrospray ionisation ($ESI^+$) mass spectra were obtained for urine samples collected from a horse given 300 mg pyrilamine iv. The samples were filtered by centrifugation (90 min at 1200 *g*) through a 3,000 molecular weight cut-off filter (Amicon, Massachusetts, USA). The filtrate was diluted 1:10 with 50% acetonitrile (with 0.05% formic acid) in water. The sample was infused into the

electrospray port of the MS/MS at 0.6 ml/h via a Harvard syringe pump equipped with a 500 µl Hamilton gas-tight syringe. The MS/MS was tuned for positive ion spectra by direct infusion of 10 ng/µl pyrilamine in 50% acetonitrile (with 0.05% formic acid) in water. Spectra were analysed with the aid of Quadtech Mass Spec Calculator software (ChemSW Inc, California, USA).

### *Pyrilamine/O-desmethylpyrilamine analysis by GC/MS*

***Enzymatic hydrolysis of urine:*** Standard solutions of pyrilamine, O-DMP and albuterol were prepared in methanol. Extraction standards were prepared by addition of a known amount of a pyrilamine and O-DMP solution to blank urine samples. A known amount of an albuterol standard (20 µl of 10 µg/ml methanol solution) was added to each sample, standard and blank as an internal standard.

The urine samples, standards and blanks were placed in culture tubes. To each sample was added 1 ml ß-glucuronidase solution (Type H-5, from *Helix pomatia*, 5000 units/ml, Sigma Chemical Co., Missouri, USA) and 2 ml of 1 M sodium acetate buffer (pH 5). The samples were mixed briefly by vortex and incubated in a water bath at 37°C overnight (12–16 h). After cooling, the pyrilamine, O-DMP and albuterol supplements were added, the samples were sonicated for 90 s, sodium phosphate buffer (2 ml of 0.1 M, pH 6) was added and the sample pH was adjusted to 6 ± 0.5 with 1 M sodium hydroxide or 1 M hydrochloric acid.

***Preparation of serum samples:*** The serum samples, standards and blanks were placed in culture tubes. The pyrilamine, O-DMP and albuterol supplements were added, sodium phosphate buffer (2 ml of 0.1 M, pH 6) was added and the sample pH was adjusted to 6 ± 0.5 with 1 M sodium hydroxide or 1 M hydrochloric acid.

***Extraction/derivatisation:*** Clean Screen SPE columns (United Chemicals Technologies-Worldwide Monitoring, Pennsylvania, USA) were conditioned by sequentially adding methanol (3 ml), water (3 ml) and 1 ml of 0.1 M sodium phosphate buffer (pH 6). The samples were then loaded and the column was washed sequentially with water (2 ml), 1 M acetic acid (2 ml), and methanol (4 ml). The column was eluted with 3 ml dichloromethane/isopropanol/ammonium hydroxide (78:20:2). The eluent was evaporated to dryness under a stream of nitrogen gas (<40°C). For derivatisation, each sample was dissolved in 40 µl BSTFA with 1% TMS, vortexed for 15 s and incubated at 75°C for 45 min.

**TABLE 1: ELISA cross-reactivity**

| Drug | $I_{50}$ (ng/ml) |
|---|---|
| Pyrilamine | 0.065 |
| Tripelennamine | 0.35 |
| O-desmethylpyrilamine | 0.54 |
| Chlorpheniramine | 35 |
| Pheniramine | 75 |
| Diphenhydramine | 1000 |

Buffer solutions of pyrilamine, its metabolite O-desmethylpyrilamine and other structurally related compounds were analysed by the Neogen pyrilamine ELISA. Reactivity of the substance with the ELISA antibody is expressed as the concentration at 50% maximum activity ($I_{50}$)

***Instrumentation:*** Each derivatised sample was transferred to an autosampler vial. A Hewlett-Packard Model 6890 GC equipped with a Model 5972A mass selective (MS) detector (Hewlett-Packard, California, USA) was used for GC analysis. The column was a J&W DB-5MS, 30 m x 250 µm x 0.25 µm (Supelco, Pennsylvania, USA). The carrier gas was helium with a 1 ml/min flow. A volume of 1 µl was injected in splitless mode at an injector temperature of 250°C. Initial oven temperature was 70°C (held 2 min), ramping at 20°C/min to 280°C (held 12 min). Total run time was 24.5 min, and the MSD transfer line temperature was 280°C. The MS was run in SIM mode with data collected from 3–24.5 min. Ion 121 m/z (pyrilamine) and ion 179 m/z (O-DMP-mono-TMS) were utilised along with ion 369 m/z (albuterol-tri-TMS, internal standard) for quantification.

## Results

### *ELISA cross-reactivity and standard curves*

A pyrilamine ELISA standard curve indicated that addition of 0.065 ng pyrilamine/ml produced 50% inhibition of colour formation. Higher pyrilamine concentrations increased inhibition in a sigmoidal manner, with complete inhibition of the ELISA test occurring at 0.1 ng/ml pyrilamine. This ELISA also reacts well with O-DMP ($I_{50}$= 0.54 ng/ml), the principal metabolite of pyrilamine and tripelennamine, another antihistamine agent (150 = 0.35 ng/ml, Table 1). There was minimal detectable cross-reactivity with other antihistamine agents chlorpheniramine, pheniramine and diphenhydramine.

### *ELISA quantification of 'pyrilamine equivalents' in urine and serum*

After an iv dose of 100 mg, the concentrations of pyrilamine equivalents in urine and serum reached peaks of 4.16 and 36 ng/ml at 2 and 0.5 h after injection, respectively (Table 2). Pyrilamine

**TABLE 2: ELISA pyrilamine equivalents from urine and serum**

| Time post dose (h) | Pyrilamine equivalents | |
|---|---|---|
| | Urine (µg/ml) | Serum (ng/ml) |
| 0 | 0 | 0.01 |
| 0.5 | - | 36.1 |
| 1 | - | 19.2 |
| 2 | 4.16 | 9.5 |
| 4 | 3.04 | 5.7 |
| 6 | 2.32 | 4.7 |
| 11 | 0.96 | 0.8 |
| 24 | 0.13 | 0 |
| 48 | 0.0054 | 0 |
| 69 | 0.0033 | 0 |

Urine samples collected from a horse (n=1) dosed with pyrilamine, 100 mg iv, were analysed by ELISA. The apparent concentrations are expressed as pyrilamine equivalents

**TABLE 3: O-desmethylpyrilamine glucuronide by MS/MS**

| Time post dose (h) | 448 m/z intensity |
|---|---|
| 0 | 27687 |
| 1 | 362882 |
| 2 | 683227 |
| 4 | 432175 |
| 6 | 302432 |
| 8 | 188178 |
| 24 | 9938 |
| 48 | 11023 |

O-desmethylpyrilamine-O-glucuronide was measured by full scan direct infusion positive electrospray ionization tandem mass spectrometry. The urine samples were collected from a horse (n=1) after the administration of 300 mg pyrilamine iv. The ion of 448 mass units/charge is the pseudomolecular ion ($m+H^+$) of O-desmethylpyrilamine-O-glucuronide

equivalents were still detectable in urine at the last sampling time (69 h) and in serum at 11 h after dosing.

### *Analysis of O-desmethylpyrilamine-O-glucuronide*

Urine samples collected from a horse after the administration of 300 mg pyrilamine iv were analysed for O-DMP-O-glucuronide by full scan direct infusion positive electrospray ionisation tandem mass spectrometry. The presence of O-DMP-O-glucuronide was observed by the measurement of the ion of 448 mass units/charge (m/z), which is the pseudomolecular ion ($m+H^+$) for this compound. As there was no isolated or synthetic O-DMP-O-glucuronide available, the intensity of the ion of 448 m/z was used as a gauge of the concentration of the material. The ion 448 m/z intensity peaked at 2 h post dose and declined to pre-dose levels between 8 and 24 h (Table 3).

### *O-desmethylpyrilamine hydrolysis*

The *Helix pomatia* ß-glucuronidase hydrolysis method was optimised for enzyme concentration and incubation time (Table 4). Increasing the amount of ß-glucuronidase in the incubation mixture increased the detectable O-DMP in urine samples. The highest level added (5,000 units) resulted in the highest observed O-DMP concentration. The optimal time of incubation (at 37°C) was observed to be 12 h with at least 75% of maximum between 6 h and 16 h.

### *GC/MS quantification*

The concentrations of O-DMP recovered from urine and the concentrations of pyrilamine in the serum of horses following pyrilamine administration (300 mg), both iv and orally, were determined by GC/MS-SIM (Table 5). The urine concentrations of O-DMP reached peaks (about 40 and 28 µg/ml) at 1 and 6 h following iv and oral administrations, respectively. Maximum pyrilamine concentrations in serum (about 350 and 22 ng/ml) were measured at 5 min and 1 h after treatment following iv and oral administrations, respectively.

## Discussion

The Neogen pyrilamine ELISA kit was easily able to detect pyrilamine equivalents for up to 11 h in serum and 69 h in urine following administration of 100 mg pyrilamine iv. The pyrilamine equivalents are putatively composed of pyrilamine, O-DMP and possibly other metabolites. During analysis of the samples, the presence of pyrilamine or its metabolites competitively prevented the binding of pyrilamine-HRP complex to the antibody in the well. Because the HRP enzyme was responsible for the colour-producing reaction in the ELISA, the log of the concentration of pyrilamine and its metabolites (pyrilamine equivalents) in the sample was inversely related to the percentage of maximal OD of the test well. The ELISA proved very sensitive to the parent compound pyrilamine and also to the synthesised metabolite O-DMP and the analogue tripelennamine. As the Neogen ELISA Racing Kit Manual reports that this assay does not require urine sample dilution to avoid background interaction, the test should be adequately sensitive for regulatory purposes.

The full scan $ESI^+$ MS/MS analysis of directly infused filtered and diluted urine samples resulted in mass spectra that revealed the presence of O-DMP-O-glucuronide. The resultant spectra confirmed O-DMP-O-glucuronide in these samples

**TABLE 4: O-desmethylpyrilamine hydrolysis optimisation**

*ß-glucuronidase concentration*

| Enzyme units x 1,000 | Observed O-DMP (µg/ml) |
|---|---|
| 1 | 8.8 |
| 2 | 22.4 |
| 3 | 26.7 |
| 4 | 27.1 |
| 5 | 35.1 |

*Hydrolysis incubation*

| Incubation time (h) | Observed O-DMP (µg/ml) |
|---|---|
| 0 | 9.3 |
| 1 | 15.8 |
| 2 | 19.1 |
| 4 | 25.5 |
| 6 | 35.7 |
| 12 | 47.1 |
| 16 | 35.1 |
| 22 | 23.3 |

**Urine samples collected from a horse 2 h after pyrilamine administration, 300 mg iv, were analysed for O-desmethylpyrilamine (O-DMP) by GC/MS following hydrolysis with a *Helix pomatia* ß-glucuronidase preparation. The hydrolysis temperature was 37°C, and the pH was 5**

with the pseudomolecular ion of 448 m/z and reasonable qualifier ions. Without authentic O-DMP-O-glucuronide standard, quantitation of the material in the samples was not possible. However, relative quantitation in terms of the intensity of the 448 m/z ion indicated a likely time course of the glucuronide in post administration urine samples.

ß-glucuronidase hydrolysis of metabolite-glucuronide complexes in post administration urine samples have usually been performed by the authors using a *Patella vulgata* enzyme preparation with incubation at 65°C for 3 h (Harkins *et al.* 1998). However, the synthesised O-DMP was found to be relatively unstable under these conditions. After incubation for 3 h at 65°C only 20% O-DMP remained (Harkins *et al.* 2000). Joujou-Sisic *et al.* (1996) have described ß-glucuronidase hydrolysis of isoxsuprine-glucuronide using an enzyme preparation isolated from *Helix pomatia* and incubation at 37°C overnight. Hydrolysis under these milder conditions, and with the *Helix pomatia* preparation, achieved a much higher concentration of recoverable O-DMP from urine samples. Our optimisation studies with this enzyme preparation indicated maximum recoverable O-DMP with an incubation time of between 6 and 16 h, and with at least 5,000 units enzyme/sample.

Pharmacokinetic parameters of pyrilamine administration were calculated using PK Analyst software (MicroMath, Utah, USA). Comparison of

**TABLE 5: Concentrations of O-DMP and pyrilamine in urine and serum, respectively, following iv and oral administration**

*Urine O-desmethylpyrilamine by GC/MS*

| Time post dose (h) | O-DMP concentration (ng/ml) | |
|---|---|---|
| | iv administration | oral administration |
| 0 | 0.2 | 0.3 |
| 1 | 40439 | 132 |
| 2 | 30224 | 6285 |
| 4 | 24443 | 23396 |
| 6 | 30662 | 28438 |
| 8 | 20676 | 25267 |
| 24 | 1241 | 1524 |
| 48 | 139 | 95.4 |
| 72 | 16.2 | 13.9 |
| 96 | 5.6 | 9.8 |
| 168 | 4.4 | 3.2 |

*Serum pyrilamine by GC/MS*

| Time post dose (h) | Pyrilamine concentration (ng/ml) | |
|---|---|---|
| | iv administration | oral administration |
| 0 min | 0 | 0.3 |
| 5 min | 352 | - |
| 10 min | 267 | - |
| 15 min | 245 | - |
| 30 min | 187.4 | - |
| 1 h | 130.7 | 22.4 |
| 2 h | 75.8 | 7.7 |
| 4 h | 26.5 | 1.9 |
| 8 h | 6.3 | 0.6 |
| 24 h | 0 | 0 |
| 48 h | 0 | 0 |

**Urine and serum samples collected from one horse (n=1) after iv pyrilamine administration (300 mg) and from another horse (n=1) after oral pyrilamine administration (300 mg) were analysed for O-desmethylpyrilamine (a) and pyrilamine (b), respectively, by GC/MS SIM. The samples were extracted by a solid phase method**

the area under the curve from 0 to infinity ($AUC_{0\text{-}inf}$) following oral and iv administration suggested relatively low (12%) bioavailability of pyrilamine in horses. The urine concentrations of recoverable O-DMP after iv and oral administrations were essentially equivalent at 6 h post dose. This suggests rapid oral absorption and extensive first-pass metabolism followed by enterohepatic circulation of this agent.

Pyrilamine is used widely in equine medicine in the USA and oral preparations of pyrilamine, in combination with other agents, are widely marketed to horsemen. Consistent with this widespread use, pyrilamine or its major urinary metabolite, O-DMP, are identified commonly in post race urine samples. For example, in one racing jurisdiction in USA, when the number of ELISA tests performed on each post race sample was increased from 3 to about 30, the number of post race

identifications of pyrilamine increased substantially.

Given the high sensitivity of the Neogen ELISA test for pyrilamine and O-DMP, it seems likely that many, if not most, of these identifications could represent detection of sub-therapeutic or trace residues of these agents at relatively long periods after the last administration of pyrilamine. Consistent with this belief, the data presented here show that pyrilamine, as its major urinary metabolite, O-DMP, remains detectable by GC/MS in post administration samples for up to one week after a single oral or iv dose. If the dose was large, or if treatment schedule was prolonged, pyrilamine and or its major urinary metabolites would presumably remain detectable at trace levels for significantly longer than one week after the last administration.

Beyond this, it is of interest to note that the oral bioavailability of pyrilamine is relatively poor, in the order of 12%, based on these preliminary data. Based on this finding, it is even less likely that oral administration of pyrilamine will be associated with significant pharmacological or performance influencing effects in racehorses. These factors should be borne in mind by horsemen and veterinarians when pyrilamine is recommended for oral administration to horses, where the modest likelihood of a significant pharmacological or therapeutic effect must be balanced against a relatively long period for which the horse's urine may contain detectable traces of pyrilamine or O-DMP. Similarly, the potential for pyrilamine or O-DMP to be retained in horse urine at trace levels for relatively long periods after last administration of this agent should be considered by regulatory or veterinary authorities reviewing trace level evidence for presence of pyrilamine or O-DMP in post race samples.

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## References

Bosken, J.M., Lehner, A.F., Hunsucker, A., Harkins, J.D., Woods, W.E., Karpiesiuk, W., Carter, W.G., Boyles, J., Fisher, M. and Tobin, T. (2000) Direct MS-MS identification of isoxsuprine-glucuronide in post administration equine urine. *Can. J. vet. Res.* **64**, 112-116.

Douglas, W.W. (1985) Histamine and 5-hydroxytryptamine (serotonin) and their antagonists. In: *The Pharmacological Basis of Therapeutics*. Eds: A.G. Gilman, L.S. Goodman, K. Rall, F. Murad. MacMillan Publishing, New York, pp 605-638.

Harkins, J.D., Mundy, G.D., Woods, W.E., Lehner, A.F., Karpiesiuk, W., Rees, W.A., Dirikolu, L., Bass, S., Carter, W.G., Boyles, J. and Tobin, T. (1998) Lidocaine in the horse: Its pharmacological effects and their relationship to analytical findings. *J. vet Pharmacol. Therap.* **21**, 462-476.

Harkins, J.D., Sams, R.A., Woods, W.E., Lehner, A.F., Karpiesiuk, W., Hughes, C., Gates, R.S., Fisher, M. and Tobin, T. (2000) Pharmacokinetics, bioavailability and serum and urine detection of pyrilamine and its major urinary metabolite in the horse. In preparation.

Joujou-Sisic, K., Andren, P.E., Bondesson, U., Fredriksson, E., Larsson, C.I., Kallings, P. and Persson, S.G.B. (1996) A pharmacokinetic study of isoxsuprine in the horse. *Proc. 11th int. Conf. racing Anal. Vet.* Eds: D.E. Auer and E. Houghton. R&W Publications, Newmarket, pp 453-458.

Korfmacher, W.A., Holder, C.L., Betowski, L.D. and Mitchum, R.K. (1988) Characterization of doxylamine and pyrilamine metabolites via thermospray/mass spectrometry and tandem mass spectrometry. *Biomed. environ. Mass. Spect.* **15**, 501-508.

Rees, W.A., Kwiatkowski, S., Stanley, S.D., Granstrom, D.E., Yang, J-M., Gairola, C.G., Glowczyk, J., Woods, W.E. and Tobin, T. (1996) The development and characterization of an ELISA for *trans-3'*-hydroxycotinine, a biomarker for mainstream and sidestream smoke exposure. In: *Environmental Toxicology and Risk Assessment: Biomarkers and Risk Assessment*. Eds: D. A. Bengston and D. S. Henshel (5th Vol), *Am. Soc. Testing Materials*, Philadelphia, pp 149-162.