Chromatographia
June 2012, Volume 75, Issue 11-12, pp 635-643
Date: 08 Apr 2012

Identification of Acepromazine and Its Metabolites in Horse Plasma and Urine by LC–MS/MS and Accurate Mass Measurement

Abstract

Acepromazine maleate (Sedalin®) was administered orally to six thoroughbred horses at a dose of 0.15 mg kg^{-1}. Urine and blood samples were collected up to 412 h post-administration. Plasma and urine were hydrolysed; plasma samples were then processed using liquid–liquid extraction and urine samples using solid-phase extraction. A sensitive tandem mass spectrometric method was developed in this study, achieving a lower limit of quantification for acepromazine of 10 pg mL^{-1} in plasma and 100 pg mL^{-1} in urine. Acepromazine, hydroxyethylpromazine, hydroxyacepromazine, hydroxyethylpromazine sulphoxide, hydroxyethylhydroxypromazine, dihydroxyacepromazine and dihydroxyhydroxyethylpromazine were detected in the post-administration samples. The parent drug and its metabolites were identified using a combination of UPLC–MS/MS and accurate mass measurement. Separation of the structural isomers hydroxyethylpromazine sulphoxide and hydroxyethylhydroxypromazine was another significant outcome of this work and demonstrated the advantages to be gained from investing in chromatographic method development.

Chromatographia (2012) 75:635–643
DOI 10.1007/s10337-012-2234-4

ORIGINAL

Identification of Acepromazine and Its Metabolites in Horse Plasma and Urine by LC–MS/MS and Accurate Mass Measurement

M. E. Wieder · B. P. Gray · P. R. Brown · S. Hudson · C. M. Pearce · S. W. Paine · L. Hillyer

Received: 25 November 2011 / Revised: 16 March 2012 / Accepted: 22 March 2012 / Published online: 8 April 2012

Abstract Acepromazine maleate (Sedalin®) was administered orally to six thoroughbred horses at a dose of 0.15 mg kg^{-1}. Urine and blood samples were collected up to 412 h post-administration. Plasma and urine were hydrolysed; plasma samples were then processed using liquid–liquid extraction and urine samples using solid-phase extraction. A sensitive tandem mass spectrometric method was developed in this study, achieving a lower limit of quantification for acepromazine of 10 pg mL^{-1} in plasma and 100 pg mL^{-1} in urine. Acepromazine, hydroxyethylpromazine, hydroxyacepromazine, hydroxyethylpromazine sulphoxide, hydroxyethylhydroxypromazine, dihydroxyacepromazine and dihydroxyhydroxyethylpromazine were detected in the post-administration samples. The parent drug and its metabolites were identified using a combination of UPLC–MS/MS and accurate mass measurement. Separation of the structural isomers hydroxyethylpromazine sulphoxide and hydroxyethylhydroxypromazine was another significant outcome of this work and demonstrated the advantages to be gained from investing in chromatographic method development.

Keywords LC–MS/MS · LTQ Orbitrap · UPLC · Acepromazine · HEPS

M. E. Wieder (✉) · B. P. Gray · P. R. Brown · S. Hudson · C. M. Pearce
HFL Sport Science, Cambridgeshire, UK
e-mail: mwieder@hfl.co.uk

S. W. Paine · L. Hillyer
British Horseracing Authority, London, UK

Introduction

Acepromazine [10-(3-dimethylaminopropyl)phenothiazin-2-yl methyl ketone] (Fig. 1) is a phenothiazine-derived, anti-psychotic drug, frequently used as a sedative in animals when mild sedation is required. It is also used in veterinary medicine as a pre-anaesthetic agent for surgical procedures, enabling a lower dose of anaesthetic to be used and, hence, quicker and smoother recovery [1]. The presence of pharmacologically significant concentrations of acepromazine in racehorses on race days is prohibited by the British Horseracing Authority (BHA) and many other horseracing and equestrian sport authorities [2]. In this study, the detection of acepromazine and its metabolites in post-administration samples was further investigated to better advise on the withdrawal of this medication ahead of competition.

Previous studies have identified acepromazine and its metabolites in the urine and blood plasma of various mammalian species following administration. Elliot and Hale et al. [3] reported the possible identification of the major unconjugated metabolite 2(1-hydroxyethyl)-promazine in human urine and blood by high-performance liquid chromatography with diode-array detection (LC–UV). Dewey et al. [4] studied the metabolism of acetylpromazine maleate in the horse following an intravenous administration. 2-(1-hydroxyethyl)promazine sulphoxide was identified as the major unconjugated metabolite by TLC, UV, GC–MS and NMR. 2-(1-hydroxyethyl)promazine, conjugated 7-hydroxyacetylpromazine, and 2-(1-hydroxyethyl)-7-hydroxy-promazine were also isolated and identified. Bertone et al. reported that acepromazine produced a number of metabolites that could be detected in horse urine. Since the duration of effect often exceeds the time over which parent phenothiazine can be detected in

No Body Text -- translate me!
Page 2

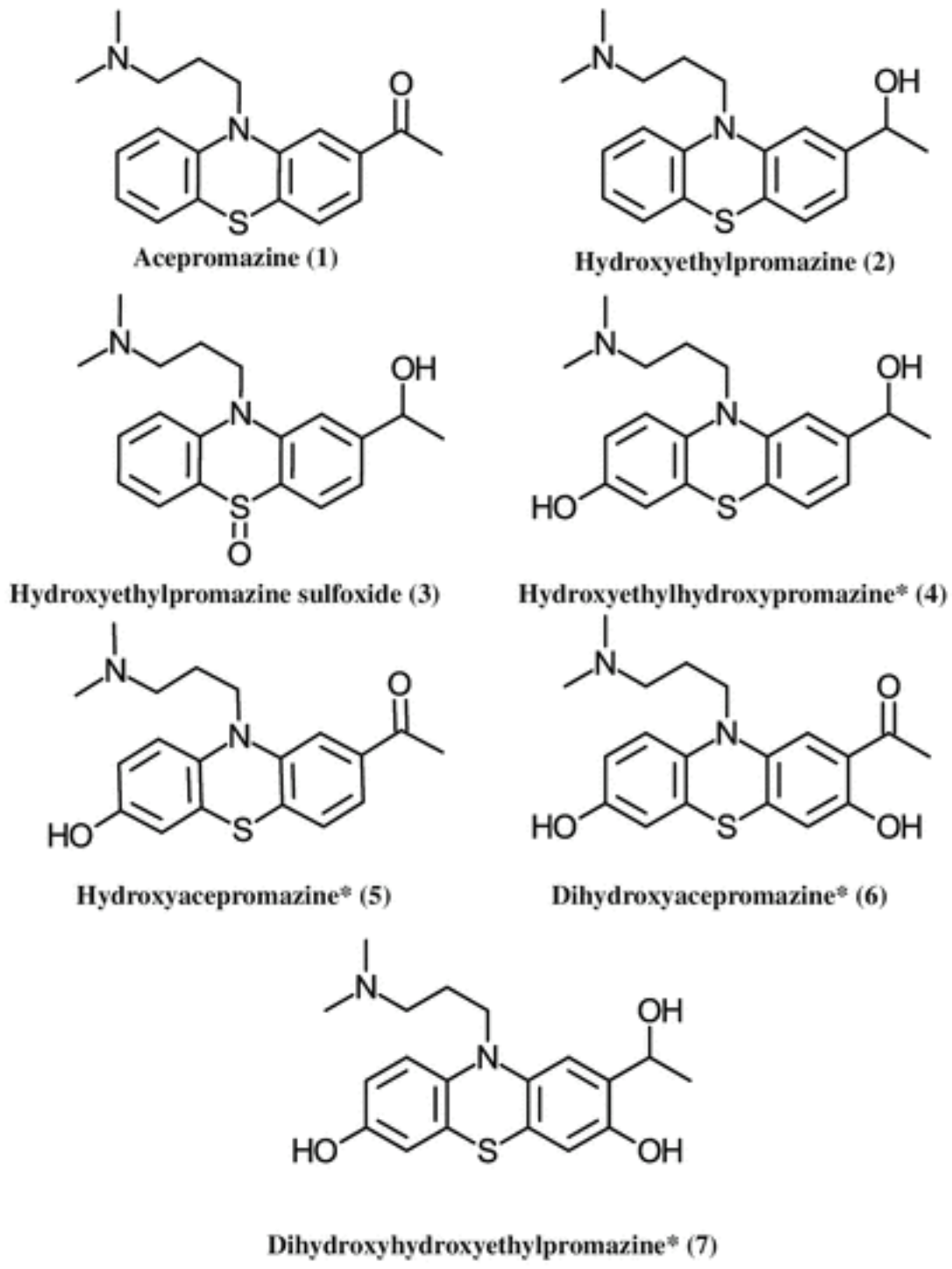


Fig. 1 Structure of acepromazine and proposed metabolites

* Proposed structure

plasma, it is possible that some metabolites retain pharmacological activity [5].

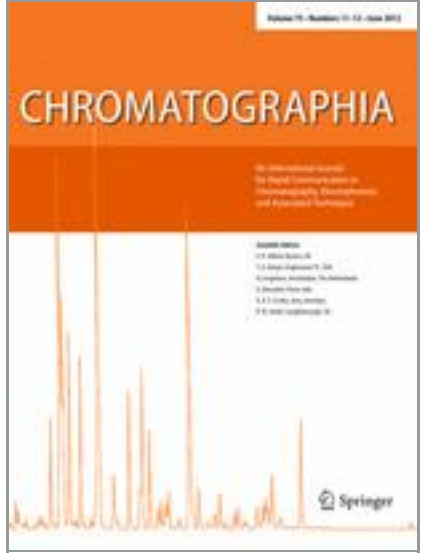
The detection of acepromazine and its metabolites in horse urine and blood plasma by liquid chromatography–tandem mass spectrometry (LC–MS/MS) using selected reaction monitoring (SRM) is reported here. The samples were also analysed on an LTQ Orbitrap® mass spectrometer to achieve accurate mass measurement of the metabolites. The primary analytical objective of the study was to characterise the metabolites present up to 17 days (412 h) after oral administration of Sedalin® (acepromazine maleate). A secondary aim was to separate the structural isomers of the known metabolites hydroxyethylpromazine sulphoxide and hydroxyethylhydroxypromazine.

Experimental

Chemicals and Reagents

Acepromazine maleate was purchased as Sedalin® from Vetoquinol UK (Buckingham, UK) for the administration study. An acepromazine reference standard was purchased from USP (Rockville, USA). Hydroxyethylpromazine was purchased from Fleet Bioprocessing (Wintney, UK), and D4-hydroxyethylpromazine sulphoxide and hydroxyethylpromazine sulphoxide were purchased from Frontier BioPharm (Richmond, Kentucky, USA). Acetic acid, chloroform, ethyl acetate, methanol and propan-2-ol were obtained from Fisher Scientific (Leicestershire, UK).

No Body Text -- translate me!



Article Metrics

2 Citations

Related Content

Loading related content...

References (14)

1. Kalhoro, AB (2006) Sedative effects of acepromazine and xylazine in horses: a comparative study. Pak J Biol Sci 9: pp. 72-75 CrossRef
2. Toutain PL, Lassourd V (2002) Pharmacokinetic/pharmacodynamic approach to assess irrelevant plasma or urine drug concentration in post-competition samples for drug control in the horse. Equine Vet J 34(3):242–249
3. Elliot, SP, Hale, KA (1999) A previously unidentified acepromazine metabolite in humans: implications for the measurement of acepromazine in blood. J Anal Toxicol 23: pp. 367-371
4. Dewey, EA, Maylin, GA, Ebel, JG, Henion, JD (1980) The metabolism of promazine and acetylpromazine in the horse. Drug Metab Dispos 1: pp. 30-36
5. Bertone J, Horspool LJI (2004) Equine clinical pharmacology. Elsevier Health Sciences, p 272
6. Weir, JJ, Sanford, J (1969) Metabolism and excretion of promazine by the horse. J Pharm Pharmacol 21: pp. 169-175 CrossRef
7. Dewey EA, Maylin GA (1983) Metabolism of propionylpromazine in the horse. In: Proceedings of the 5th international conference on the control of drugs in racehorses, vol 5, pp 196–199
8. Park J, Shin YOS, Choo H-YP (1989) Metabolism and pharmacokinetic studies of propionylpromazine in horses. J Chromatogr 489:313–321
9. Salzman, NP, Brodie, BB (1956) Physiological disposition and fate of chlorpromazine and a method for its estimation in biological material. J Pharmacol Exp Ther 118: pp. 46-54
10. Forrest, IS, Forrest, FM, Berger, M (1958) Free radicals as metabolites of drugs derived from phenothiazine. Biochim Biophys Acta 29: pp. 441-442 CrossRef
11. Forrest, IS, Green, DE (1972) Phenothiazines: metabolism and analytical detection. J Forensic Sci 17: pp. 592-617
12. Smyth, WF (2003) Electrospray ionisation mass spectrometric behaviour of selected drugs and their metabolites. Anal Chim Acta 492: pp. 1-16 CrossRef
13. Scarth, JP, Spencer, HA, Timbers, SE, Hudson, SC, Hillyer, LL (2010) The use of in vitro technologies coupled with high resolution accurate mass LC–MS for studying drug metabolism in equine drug surveillance. Drug Test Anal 2: pp. 1-10
14. Zhao X-G, Ma J, Feng H, Wu J, Gu Z-M (2004) Loss of Hydroxyl radical (·OH) in CAD MS/MS for identification of oxidised drug metabolites. Xenobiotic Laboratory Inc., New Jersey

About this Article

Title
Identification of Acepromazine and Its Metabolites in Horse Plasma and Urine by LC–MS/MS and Accurate Mass Measurement
Journal
Chromatographia
Volume 75, Issue 11-12 , pp 635-643

Cover Date
2012-06-01
DOI
10.1007/s10337-012-2234-4
Print ISSN
0009-5893
Online ISSN

1612-1112
Publisher
Springer-Verlag
Additional Links

- Register for Journal Updates
- Editorial Board
- About This Journal
- Manuscript Submission

Topics

- Pharmacy
- Proteomics
- Laboratory Medicine
- Chromatography

Keywords

- LC–MS/MS
- LTQ Orbitrap
- UPLC
- Acepromazine
- HEPS

Industry Sectors

- Pharma
- Materials & Steel
- Automotive
- Chemical Manufacturing
- Biotechnology
- Electronics
- IT & Software
- Telecommunications
- Consumer Packaged Goods
- Energy, Utilities & Environment
- Aerospace
- Oil, Gas & Geosciences
- Engineering

Authors

- M. E. Wieder [(1)]
- B. P. Gray [(1)]

- P. R. Brown [1]
- S. Hudson [1]
- C. M. Pearce [1]
- S. W. Paine [2]
- L. Hillyer [2]

Author Affiliations

- 1. HFL Sport Science, Cambridgeshire, UK
- 2. British Horseracing Authority, London, UK