

Perindopril

DETECTION AND CONFIRMATION IN EQUINE URINE

by

**Truesdail Laboratories, Inc.
14201 Franklin Avenue
Tustin, CA 92780**

For

The Testing Integrity Program

Method Author: Christopher A. Natrass

**Contact person: Chris Natrass
Phone: (714) 730-6239 x650
Fax: (714) 730-6462
E-mail: ChrisN@Truesdail.com**

Abstract

Perindopril, ((2S,3aS,7aS)-1-[(2S)-2-[[[(1S)-1-(Ethoxycarbonyl)butyl]amino]-1-oxypropyl]octahydro-1H-indole-2-carboxylic acid, is distributed under the trade name ACEON® in the form of perindopril erbumine, the tert-butylamine salt of perindopril.

The screening technique is an acidic extraction into organic solvent, followed by liquid chromatography / mass spectroscopy. Confirmation uses the same technique as screening, with the addition of appropriate quality control measures to be determined by individual laboratories.

Scope

The following method is proposed for liquid chromatography / mass spectroscopy (LC/MS²) detection and confirmation of perindopril in equine urine. Based on the suspected fragmentation pattern, LC/MS scan parameters for perindoprilat, the active metabolite of perindopril in humans, are incorporated in this method. However, perindoprilat was not detected in the equine post administration urine tested. The LC/MS detection limit for perindopril is about 1 ng/mL in equine urine.

Principle

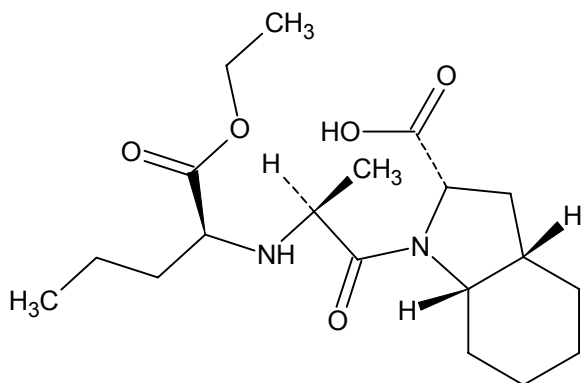
An acidified (pH4) liquid-liquid extraction procedure into 3:1 dichloromethane : isopropyl alcohol is used for preparation of equine urine specimens prior to screening by LC/MS. The usual principals of liquid-liquid extraction apply. It should be noted that solid phase extraction (acidic fraction) yielded similar recoveries.

Standards

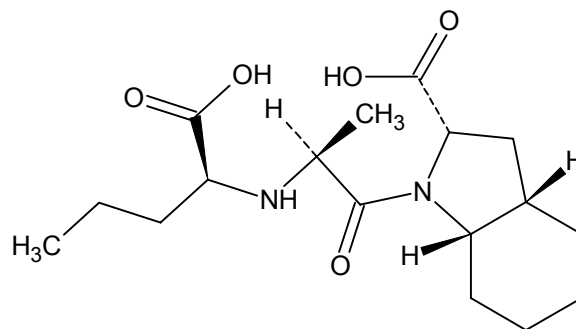
Perindopril is commercially available as ACEON® tablets. A ~1.0 mg/ml stock solution was used in developing this procedure and was prepared by dissolving five 2.0 milligram ACEON® tablet in 10.0 mL DI water and then removing the starch by centrifugation and filtration. Enalapril was purchased as Vasotec® 20 mg tablets from a local pharmacy. Enalapril is commercially available (Sigma and others) and was prepared in methanol in 1.0 milligram per milliliter solutions. Enalaprilat (generic) was purchased as an IV solution from a local pharmacy. The injectable solution comes at a concentration of 1.25 mg/mL and was used as is.

Limitations

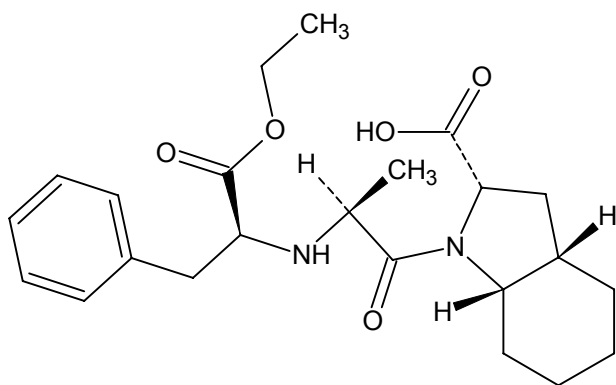
Store stock solutions under nitrogen to reduce oxidation. Exposure to light may also cause degradation.



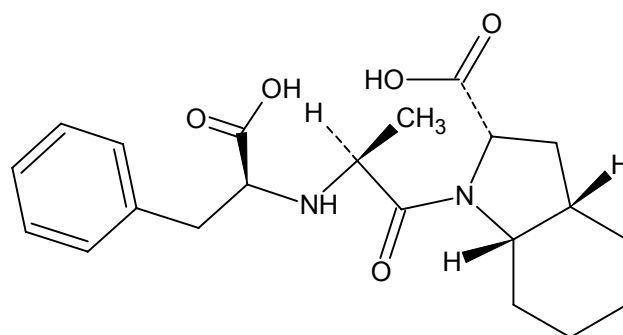
Perindopril



Perindoprilat



Enalapril



Enalaprilat

METHODOLOGY

References

Extraction method:

NASRC Quality Assurance Program (1982-1988)

ARCI Quality Assurance Program (1988-1995)

The extraction method below is a modification of a method provided by the above two programs.

Extraction and Screening

APPARATUS

- 16 x 100 mm screw-top test tubes with caps
- 16 x 125 mm screw-top test tubes with caps
- Automatic pipettor with 10 ml graduated pipette
- Rotorack mixer for test tubes
- Centrifuge
- Vacuum aspiration apparatus
- Water bath
- Liquid chromatograph / mass spectrometer system (LC/MS) with MS² capability.
- HPLC column (*Restek Ultra C18* 150 x 3.2 mm, 5µm was used)

REAGENTS

- pH 4 phosphate buffer (dissolve 770 g of potassium phosphate monobasic (KH₂PO₄) in 3.5 liters DI water)
- Dichloromethane (DCM) (technical grade that was distilled "in house" was used)
- Isopropyl alcohol (IPA) (reagent grade)
- Acetonitrile (HPLC grade)
- Ultra purified water ("Nano Water"), 17 to 18 megaohms, via NANOpure ultrapure water systems or HPLC grade water.
- Formic acid (ACS grade)

PROCEDURE

Below is a simple per sample procedure. Add internal standards and prepare spiked calibrators, controls, etc. appropriate for your particular analytical needs. Enalapril was used as the internal standard for some of the studies involved in preparing this procedure.

- 1) Add 6.0 ml equine urine to a 16 x 125 mm screw-top tube.
- 2) Make entry into "Reagent Addition" log book.
- 3) Add 2.0 ml pH 4 phosphate buffer.
- 4) Add 4.0 ml 3:1 DCM:IPA.
- 5) Cap tube, rotorack 15 minutes, centrifuge 5 minutes.
- 6) Make entry into "Sample Handling" log book.
- 7) Aspirate upper, aqueous, phase and discard.

- 8) Pour organic phase into a 16 x 100 mm screw-top tube.
- 9) Concentrate to dryness in 60°C water bath.
- 10) Add 100µL of 50:50 acetonitrile:Nano water. Vortex tube to wash down sides.
- 11) Transfer 50:50 mixture to an autosampler vial and submit for LC/MS analysis.

LC/MS SUMMARY

Analyte(s): Perindopril, Perindoprilat, Enalapril, Enalaprilat

HPLC PARAMETERS

Column: *Restek Ultra C18* 150 x 3.2 mm 5µm Temp: 30°C

Mobile phase: A Acetonitrile w/ .2% formic acid
 B Nano water w/.2% formic acid

Gradient:

Time	A	B	C	D	Flow rate
0.0	30	70			400
10	30	70			400

Retention time(s): perindopril 4.5, perindoprilat ?., enalapril 4.0, enalaprilat 2.4

MS PARAMETERS

Head type: ESI Depth: 5.5 Height: 6 Heater Temp: NA

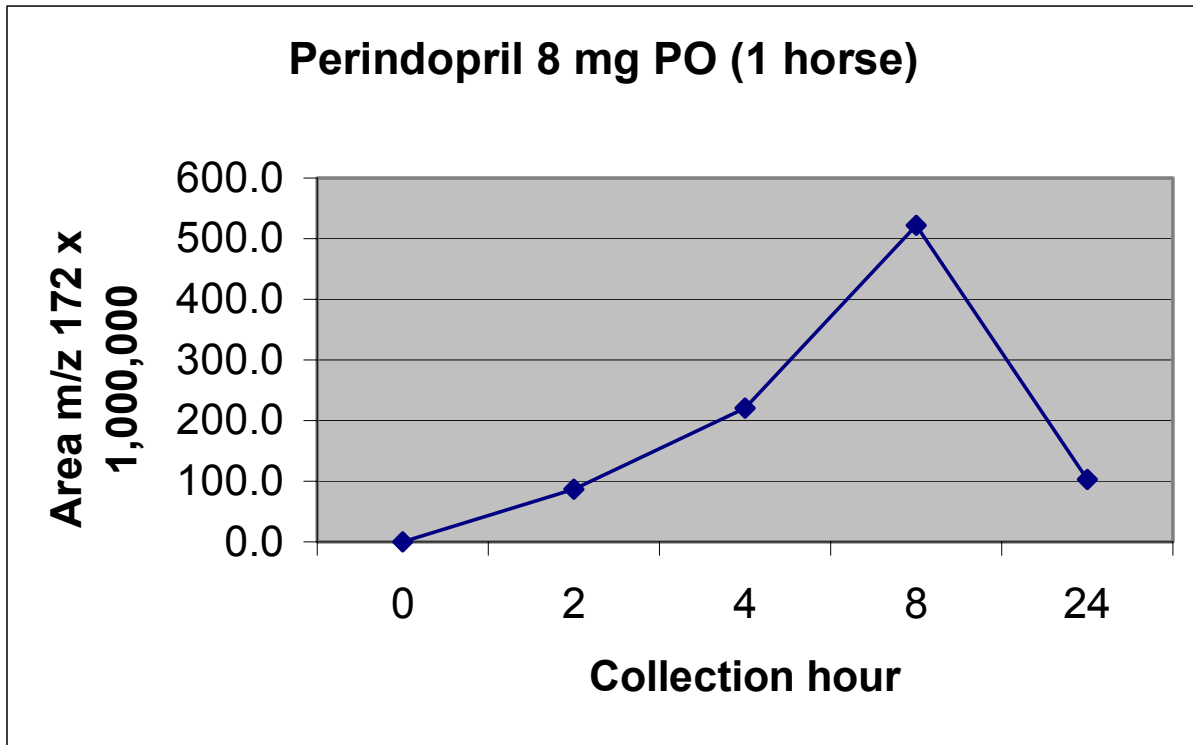
Tune File(s): perindopril esi tune (0.0 - 12.0 min)

Heated Cap Temp: 350°C Sheath gas: 90 Aux gas: 20

Scan Parameters:

Retention Time	Drug	Scan events (mode), pseudomolecular ion, Iso Width, Collision Energy, scan range	Major Ions in order of abundance
4.5 min	Perindopril	(+), 369.1, IW=2.0, CE=28, 110-375	172,295,170,369,124
?	perindoprilat	(+), 341.0, IW=2.0, CE=27, 225-405	
4.0 min	enalapril	(+), 377.1, IW=2.0, CE=28.0, 225-390	234,303,377,280
2.4 min	enalaprilat	(+), 349.0, IW=2.0, CE=27.0, 110-360	206, 303, 349, 252

PERINDOPRIL EQUINE URINE EXCRETION PROFILE



Administration urine provided by Iowa State University

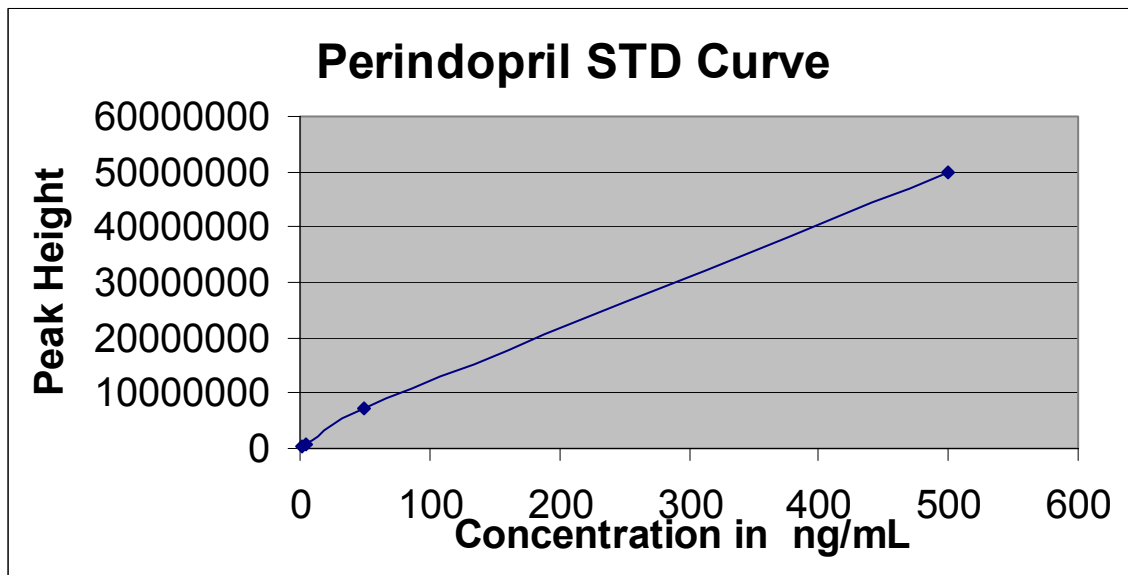
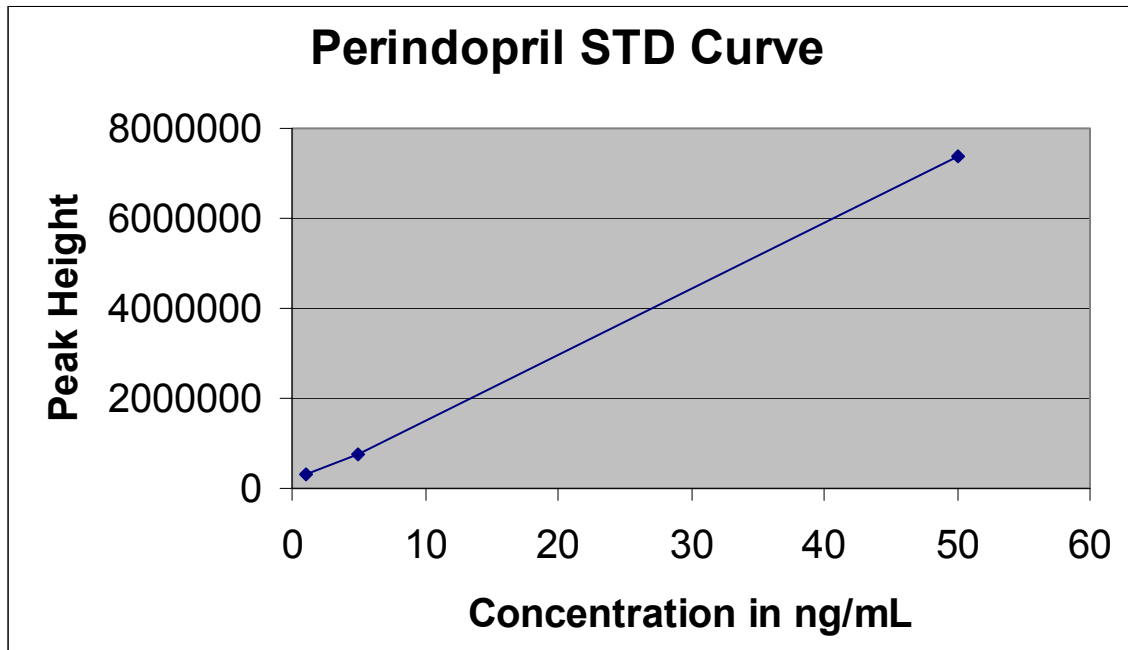
Note: No perindoprilat (the proposed metabolite) was observed; only the parent perindopril was detected.

EXTRACTION

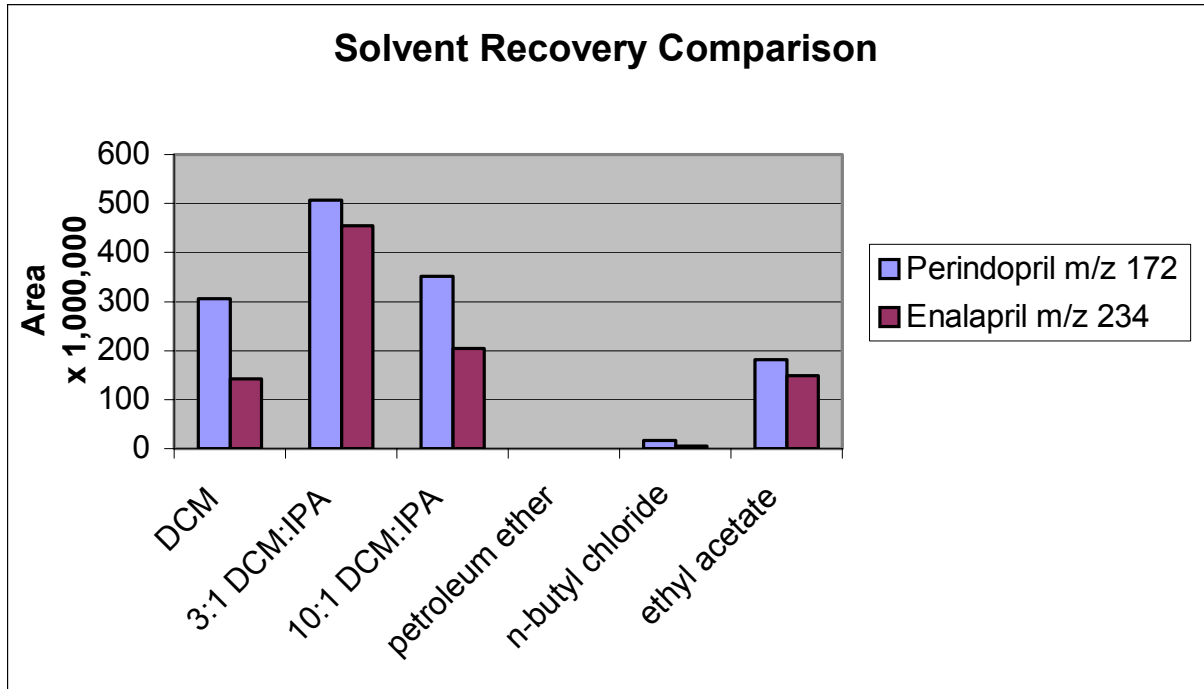
6 mL urine
2 mL pH 4 phosphate buffer
4 mL dichloromethane

Perindopril STD Curve

<u>Concentration in ng/mL</u>	<u>Peak Height</u>
1	310000
5	750000
50	7400000
500	50000000



Solvent Recovery Study



EXTRACTION

6 mL urine

2 mL pH 4 phosphate buffer

4 mL of one of the above solvents