Poster
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A Sensitive LC-MS/MS Assay for Determination of Benazepril and Benazeprilat in Dog Plasma

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INTRODUCTION

Benazepril, an angiotensin-converting enzyme inhibitor, is widely used for the treatment of cardiovascular diseases. Benazepril is converted by hepatic cleavage of the ester group to the active metabolite, benazeprilat, which have both renal and hepatic elimination. In order to support an appropriate dosing form for the treatment of heart failure and chronic renal insufficiency in companion animals, a sensitive and selective LC-MS/MS method has been developed and validated for determination of benazepril and benazerilat in dog plasma.

The structure of benazepril and benazeprilat is shown in (Figure 1).

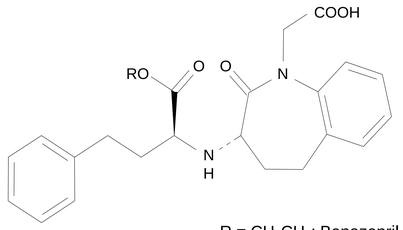


Figure 1

R = CH_2CH_3: Benazepril
R = H: Benazeprilat

METHOD

In this method, following a solid phase extraction procedure using Oasis WCX SPE cartridges (3cc, 60 mg) for sample cleanup, the extract was chromatographed using a Betasil phenyl column (20 X 2.1 mm, 3μ) and acetonitrile/methanol-based mobile phase solutions. A valve switching system is used for elimination background noise built up with injections of extracted samples. The analytes were detected by TurboIonSpray API 4000 LC-MS/MS system under positive MRM mode.

Mass Spectrometry

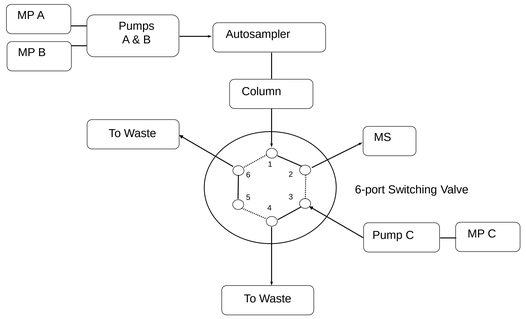
Applied Biosystem/MDS-Sciex API 4000
Polarity : Positive
Scan type : Multiple Reaction Monitoring
Benazepril : 425 → 351
Benazeprilat : 397 → 351
I.S, (Benazepril-d5): 430 → 356
I.S, (Benazeprilat-d5): 402 → 356

Extraction: Solid Phase Extraction Method

Liquid Chromatography

Shimadzu 20AD HPLC system
Autosampler : PE 200
Column: Betasil Phenyl 20 X 2.1 mm, Guard Column : Betasil Phenyl 10 X 2.1mm at 55^0 C
Mobile Phase A : 0.1% Formic Acid in Deionized water
Mobile Phase B : ACN/MeOH/Formic Acid, (50/50/0.1, V/V/V)
Mobile Phase C : ACN/10mM Ammonium Formate, pH=3.0 (90/10, V/V)

VALVE SWITCHING DESIGN



RESULTS

The quantitation ranges were 0.10 to 20.0 ng/mL and 0.50 to 100 ng/mL for benazepril and benazeprilat, respectively. Calibration curve shown bellow (Figure 2 and 3).

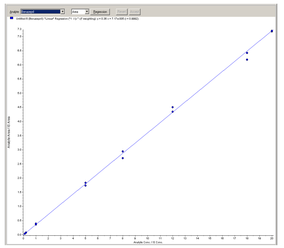


Figure 2

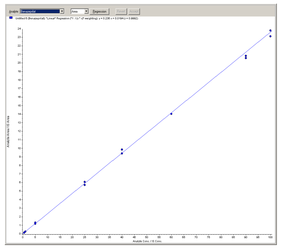


Figure 3

Figures 4,5 and 6 show typical chromatograms for LLOQ, Blank and ULOQ of benazepril and benazeprilat.

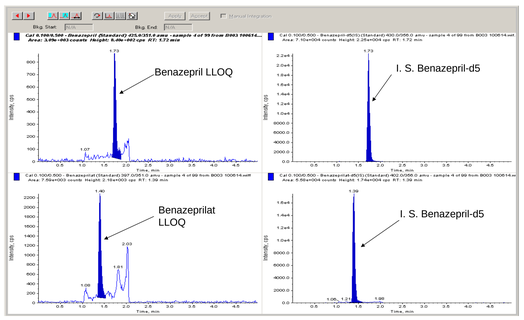


Figure 4

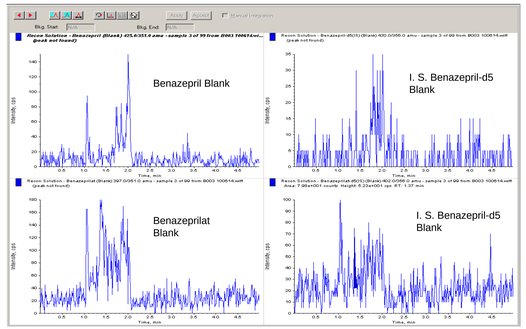


Figure 5

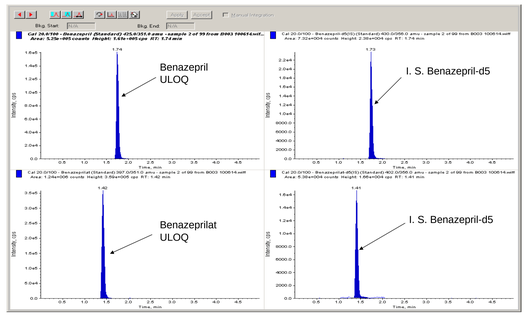


Figure 6

Table 1 and **Table 2** summarize result of inter-batch QC samples. All concentration levels demonstrate excellent precision and accuracy.

Table 1. Precision and Accuracy Results (inter-batch) for Benazepril Quality Control Samples.

	0.10 ng/mL (LLOQ)	0.30 ng/mL (Low)	10.0 ng/mL (Mid)	15.0 ng/mL (High)
n	18	18	18	18
Mean	0.0985	0.299	10.0	15.0
Precision (cv %)	10.4	4.6	1.8	2.4
Accuracy (bias %)	-1.5	-0.3	0.1	0.0

Table 2. Precision and Accuracy Results (inter-batch) for Benazeprilat Quality Control Samples.

	0.50 ng/mL (LLOQ)	1.50 ng/mL (Low)	50.0 ng/mL (Mid)	75.0 ng/mL (High)
n	18	18	18	18
Mean	0.499	1.50	50.3	74.6
Precision (cv %)	10.3	3.8	1.7	2.2
Accuracy (bias %)	-0.3	-0.3	0.6	-0.5

Matrix effect was evaluated for benazepril and benazeprilat in six different lots of blank dog plasma at concentrations of 0.30 and 1.5 ng/ml, respectively. See table 3 and 4.

Table 3. Matrix Effect for Benazepril in dog Plasma

	Avg drug area (rrf)	Avg IS area	Avg area ratio
ME 1	9514	89446	0.1064
ME 2	9392	88269	0.1064
ME 3	9752	94093	0.1036
ME 4	7981	75180	0.1062
ME 5	9324	89199	0.1045
ME 6	9688	92121	0.1052
mean	**9275**	**88051**	**0.1054**
n	**6**	**6**	**6**
SD	**655**	**6664**	**0.0011**
%CV	**7.1**	**7.6**	**1.1**

Table 4. Matrix Effect for Benazeprilat in dog Plasma

	Avg drug area (rrf)	Avg IS area	Avg area ratio
ME 1	25620	71182	0.3599
ME 2	25635	69050	0.3713
ME 3	25222	69945	0.3606
ME 4	21818	58575	0.3725
ME 5	25304	69029	0.3666
ME 6	25451	71785	0.3545
mean	**24842**	**68261**	**0.3642**
n	**6**	**6**	**6**
SD	**1490**	**4875**	**0.0070**
%CV	**6**	**7.1**	**1.9**

Table 5. Stability for Benazepril and Benazeprilat in dog Plasma

	Acceptance Criteria	Stability
Freeze/Thaw	Within 15% Change;15% CV	Stable 4 cycles
Bench-top(RT)	Within 15% Change;15% CV	Stable 8 hr
Long-term(-20^{0}C)	Within 15% Change;15% CV	Stable 90 days
Long-term(-70^{0}C)	Within 15% Change;15% CV	Stable 90 days
Autosampler	Within 15% Change;15% CV	Stable 143 hr
Refrigeration	Within 15% Change;15% CV	Stable 143 hr

The mean recovery of benazepril was determined to be 93.7%. The mean recovery of benazeprilat was determined to be 94.4%. The test for conversion of benazepril to benazeprilat was evaluated. No inter-conversion or cross talk effects were observed.

In dog plasma, 45 out of 51 samples that were analyzed as incurred repeats were within 20% of the mean of the original and repeated values for benazeprilat, whereas for benazepril 39 out of 39 incurred repeats were within 20%.

CONCLUSION

The Validated method can be demonstrated to be reliable in drug development and GLP Study.