ThP 725

LC/MS/MS Quantitation of Benzenesulfinic Acid in Rat Plasma

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Objective

Introduction

Zinc bis(benzensulphinate) (ZBS) is an alkyl aromatic zinc sulfonate complex, mainly used in pesticides, plastics, and rubber additives. The free form of zinc bis(benzensulphinate) is benzenesulfinic acid. Benzenesulfinic acid has low molecular weight and high hydrophilicity. Special LC conditions with HILIC mode was developed and an LC/MS/MS method was validated to quantitate benzenesulfinic acid in rat plasma

Method Summary

Matrix: Rat EDTA plasma

Analyte: Benzenesulfinic Acid (Zinc bis(benzensulphinate) (ZBS)
IS: Sodium p-toluenesulfinate (toluenesulfinic acid).

Method Calibration Range:
0.500-500 ng/mL

Sample Preparation:
protein precipitation by acetonitrile

HPLC conditions:
Column: Xbridge BEH Amide
Mobile phases: acetonitrile/water/Ammonium acetate

Mass spec:
API6500
Negative mode
MRM: 141/64 (analyte)
155/64 (IS)

Discussion

- Both analyte and internal standard have low molecular weight and high polarity, poorly retained in traditional reversed-phase column
- HILIC condition was used for LC separation and retention
- Ammonium acetate in the mobile phases helped to achieve better peak shape.

Validation Result

Standard Calibrators Statistics

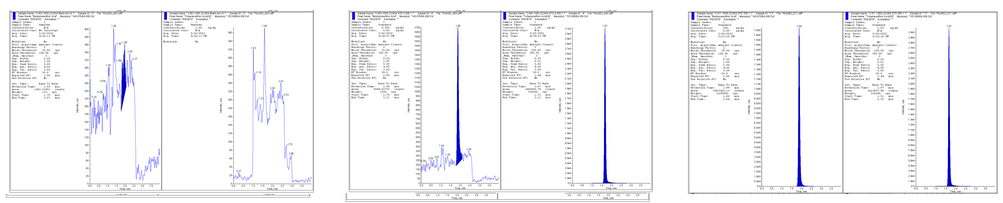
Assay Date	Analytical Run Number	STD 0.500 ng/mL	%Bias	STD 1.00 ng/mL	%Bias	STD 5.00 ng/mL	%Bias	STD 20.0 ng/mL	%Bias	STD 100 ng/mL	%Bias	STD 300 ng/mL	%Bias	STD 400 ng/mL	%Bias	STD 500 ng/mL	%Bias
13-May-2022	1	*0.310		1.10	10.0	4.90	-2.0	19.2	-4.0	98.2	-1.8	330	10.0	380	-5.0	525	5.0
		0.456	-8.8	1.10	10.0	4.66	-6.8	20.0	0.0	90.2	-9.8	300	0.0	379	-5.3	545	9.0
26-May-2022	2	0.442	-11.6	1.02	2.0	**4.16		17.5	-12.5	102	2.0	298	-0.7	351	-12.3	437	-12.6
		0.549	9.8	**1.18		5.44	8.8	21.0	5.0	**123		313	4.3	458	14.5	513	2.6
22-Jun-2022	12	0.500	0.0	0.966	-3.4	5.02	0.4	20.1	0.5	103	3.0	310	3.3	413	3.3	507	1.4
		0.538	7.6	0.884	-11.6	4.92	-1.6	19.7	-1.5	100	0.0	308	2.7	390	-2.5	492	-1.6
23-Jun-2022	13	0.471	-5.8	0.995	-0.5	5.16	3.2	19.0	-5.0	100	0.0	296	-1.3	396	-1.0	510	2.0
		0.532	6.4	0.976	-2.4	5.37	7.4	19.7	-1.5	96.7	-3.3	298	-0.7	403	0.8	506	1.2
27-Jun-2022	15	0.496	-0.8	0.973	-2.7	5.00	0.0	20.3	1.5	101	1.0	298	-0.7	410	2.5	493	-1.4
		0.515	3.0	0.986	-1.4	4.97	-0.6	19.4	-3.0	104	4.0	291	-3.0	407	1.8	501	0.2
05-Jul-2022	18	0.504	0.8	0.878	-12.2	5.05	1.0	19.8	-1.0	99.4	-0.6	312	4.0	397	-0.8	506	1.2
		0.528	5.6	0.993	-0.7	5.00	0.0	19.6	-2.0	99.5	-0.5	297	-1.0	413	3.3	513	2.6
23-Sep-2022	21	0.491	-1.8	0.980	-2.0	4.45	-11.0	20.5	2.5	98.7	-1.3	294	-2.0	400	0.0	516	3.2
		0.532	6.4	0.948	-5.2	4.90	-2.0	21.6	8.0	102	2.0	312	4.0	403	0.8	493	-1.4
13-Jan-2023	22	0.436	-12.8	0.997	-0.3	4.62	-7.6	**15.4		102	2.0	321	7.0	377	-5.8	488	-2.4
		0.567	13.4	1.01	1.0	4.95	-1.0	17.8	-11.0	107	7.0	**355		420	5.0	528	5.6
Mean		0.504		0.987		4.96		19.7		100		305		400		505	
S.D.		0.0392		0.0610		0.257		1.06		3.77		11.1		23.3		23.4	
%CV		7.8		6.2		5.2		5.4		3.8		3.6		5.8		4.6	
%Bias		0.8		-1.3		-0.8		-1.5		0.0		1.7		0.0		1.0	
n		15		15		15		15		15		15		16		16	

QC (Validation Samples VS) Accuracy and Precision Summary

A &P Parameter	VS 0.500 ng/mL	VS 1.50 ng/mL	VS 250 ng/mL	VS 375 ng/mL
Mean Conc (ng/mL)	0.495	1.38	249	363
Inter-run SD	0.0586	0.0845	18.2	22.8
Inter-run %CV	11.8	6.1	7.3	6.3
Inter-run %Bias	-1.0	-8.0	-0.4	-3.2
n	24	24	23	24

Typical Chromatograms

Blank **LLOQ** **ULOQ**



Validation Result Summary

Validation Summary	
Dilution Factor	400
Blank Selectivity	No interference with 6 different lots of matrix, plus blank with 2% hemolysis
Spiked Selectivity at LLOQ	Met criteria with 6 different lots of matrix, plus blank with 2% hemolysis
Extraction Efficiency	88% recovery for analyte
	93% recovery for internal standard
Matrix Factor	No matrix effect with 6 different lots of matrix
Long-term Stability	245 days at -20°C
Short-term Stability	24 hours at ambient condition
Freeze-thaw Stability	4 cycles at -20°C
Whole Blood Stability	2 hours at 2-8°C
Batch Reinjection Stability	66 hours at 2-8°C
Processed Sample Stability	323 hours at 2-8°C
Stock Solution Stability	75 days at 2-8°C and 23 hours at ambient condition

Conclusion

An LC/MS/MS method was validated for the quantitation of benzenesulfinic acid in rat Plasma in the range of 1.00-1000 ng/mL.

Dilution factor of 400 was validated for samples above the calibration range.

This method has been successfully used for sample analysis.

Contact Information

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