SUPPORTING INFORMATION

Development of Shotgun Metabolomic Profile analysis for canine visceral leishmaniasis using Flowthrough Pinhole Paper Spray Ionization

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Supporting information is summarized in the table below

1. **3D CAD designs for printing embossment plates**

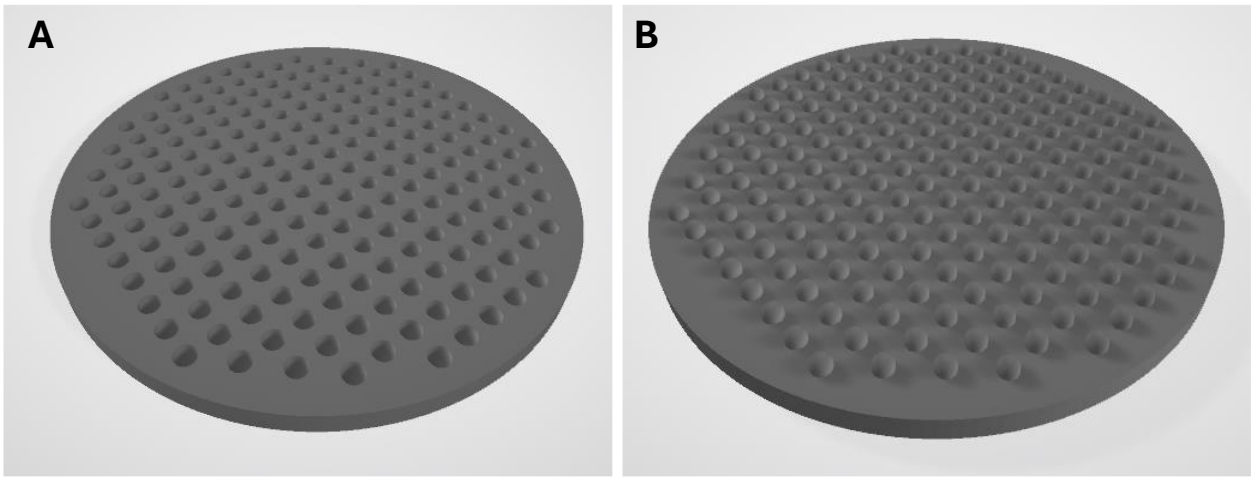

Figure S1. Computer-aided design (CAD) showing the 3D master plate molds used to print **(A)** negative embossment plates and **(B)** positive embossment plates. See STL files among the documents submitted.

2. **Illustration of Pinhole Paper Spray Mass Spectrometry**

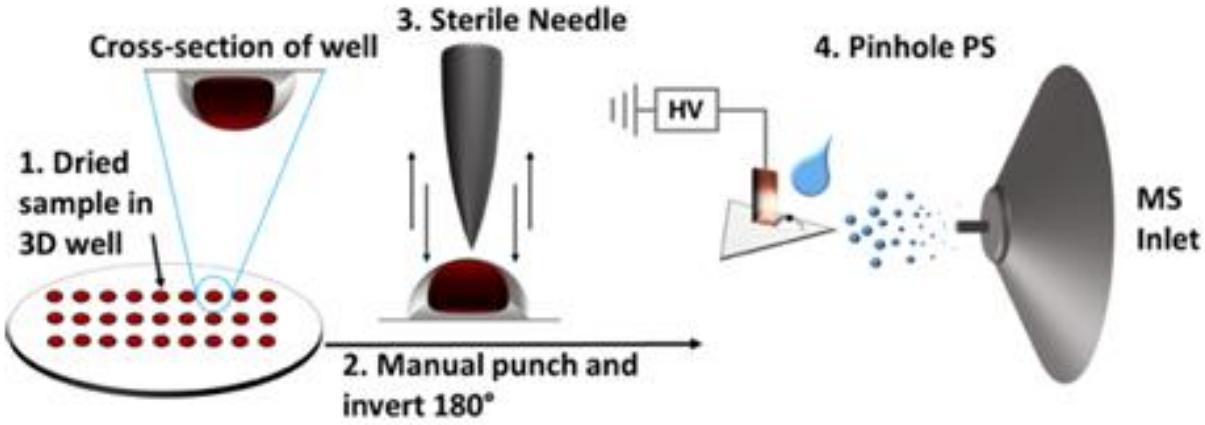

Figure S2. Graphical illustration of pinhole paper ionization including, dried samples on an embossed hydrophobic paper substrate, a sterile needle used in punching a hole at the bottom of the hydrophobic paper, and then the application of spray solvent and voltage to the hydrophobic and hydrophilic paper assembly for analysis. Instead of the pinhole paper spray in step 4, we used flow-through pinhole paper spray as shown in Figure 1. Actual photograph of the experimental setup is shown in Figure S3.

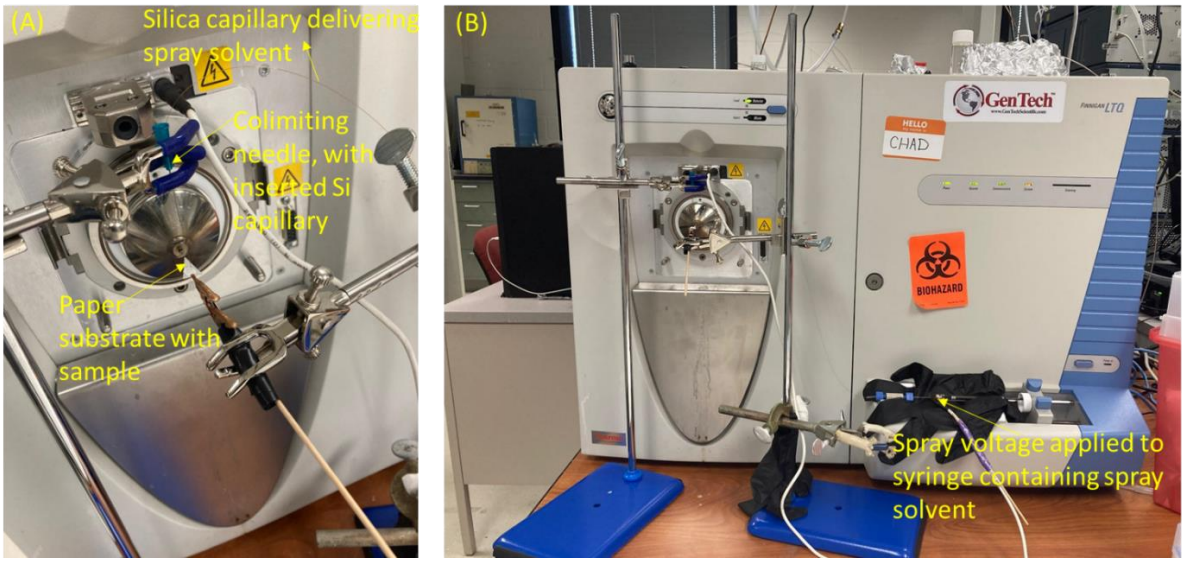

Figure S3. (**A**) Photograph showing MS inlet with the paper substrate containing the sample positioned in front, with the primary fused silica delivering the spray solvent inserted into a colimiting needle. (**B**) Photograph showing the entire MS instrument and emphasizing the syringe where the spray solvent is placed and high voltage applied.

3. Optimization of Flow-through Pinhole Paper Spray Conditions

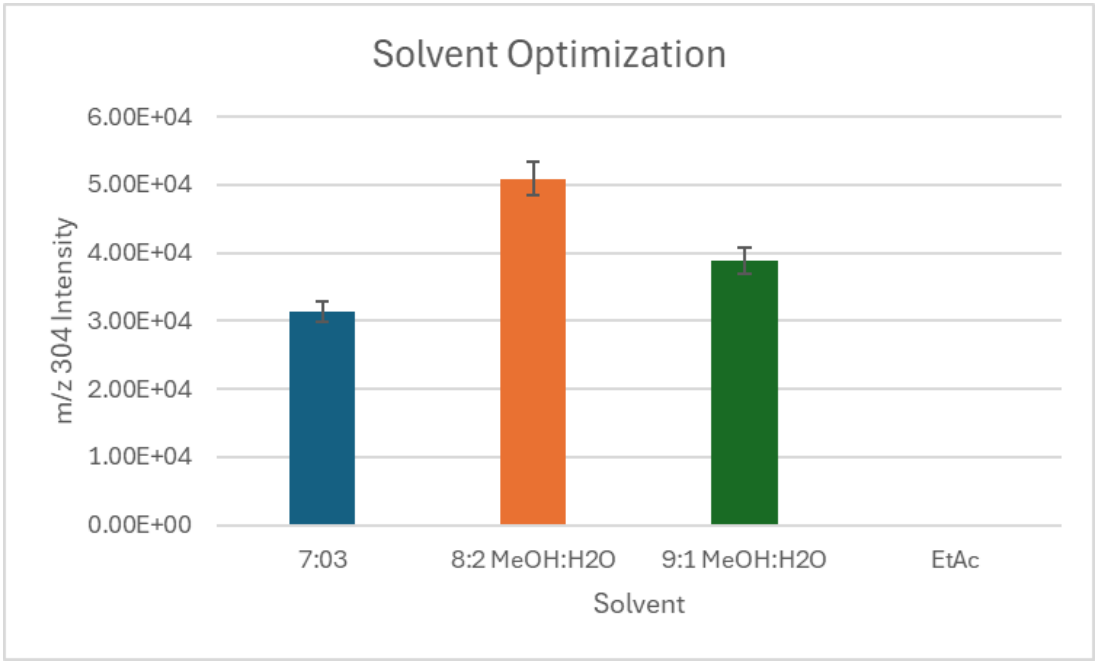

Figure S4. Effect of water composition in the spray solvent mixture on the desorption of cocaine (*m/z* 304) from the paper substrate in flow-through paper spray experiment.

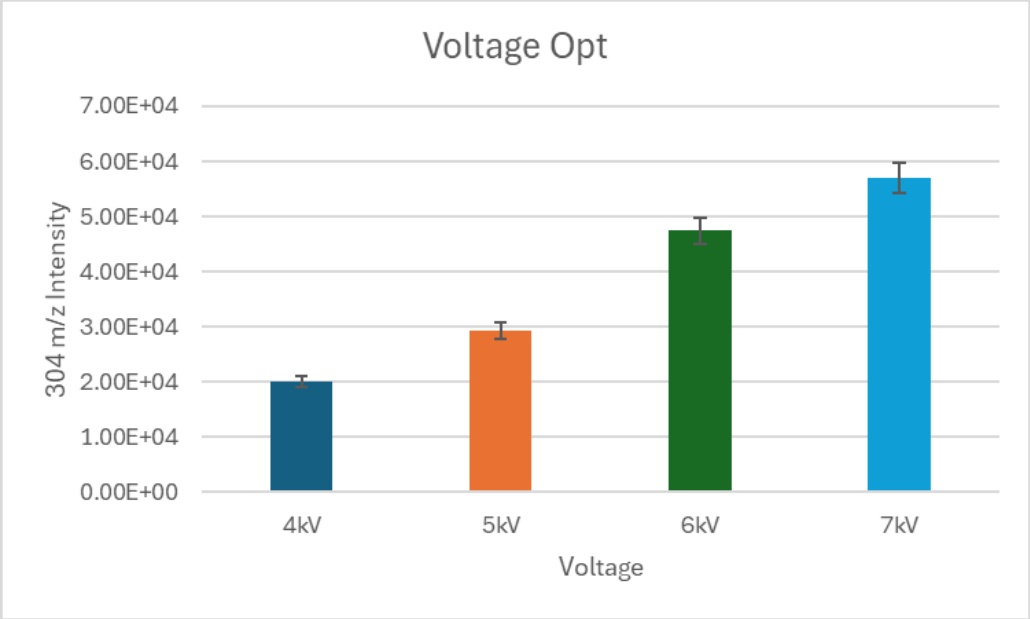

Figure S5. Effect of spray voltage at the optimized spray solvent composition of MeOH:H_2O (80:20, v/v, with 0.1% formic acid) in flow-through paper spray experiment.

4. Optimization experiment to Select the Mode of Analysis (Positive and Negative) for the Clinical Samples

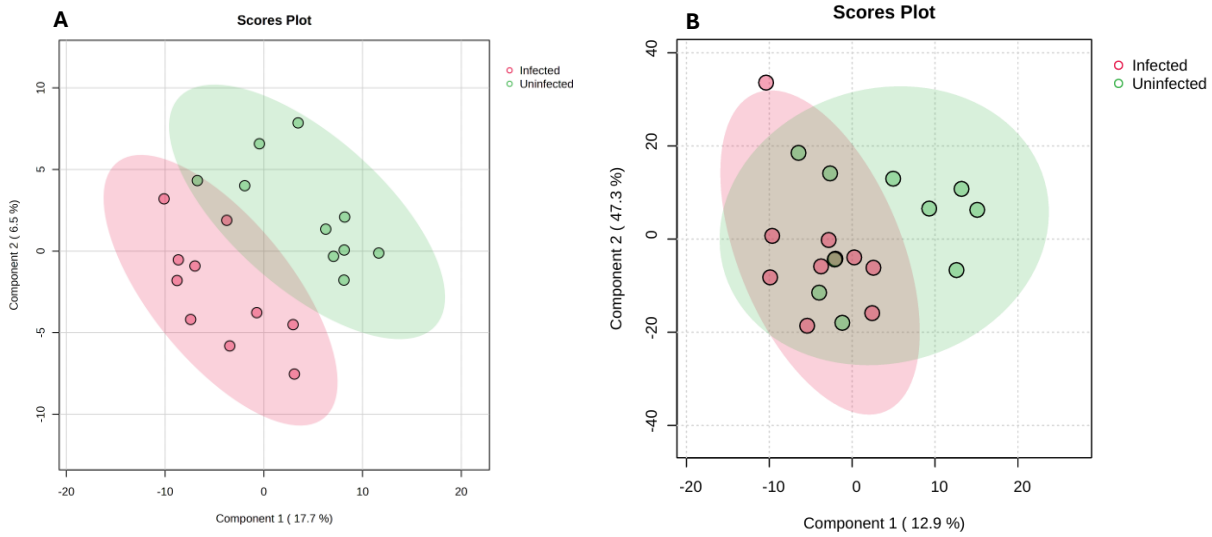

Figure S6. Partial least squares discriminant analysis of metabolite profiling from initial method optimization using flow-through pinhole paper spray MS in (**A**) positive- and (**B**) negative-ion modes. This initial optimization was performed with old canine serum samples we had in the lab, with positive-ion mode profile providing better separation than negative-ion mode.

5. Typical mass spectra recorded from canine serum samples

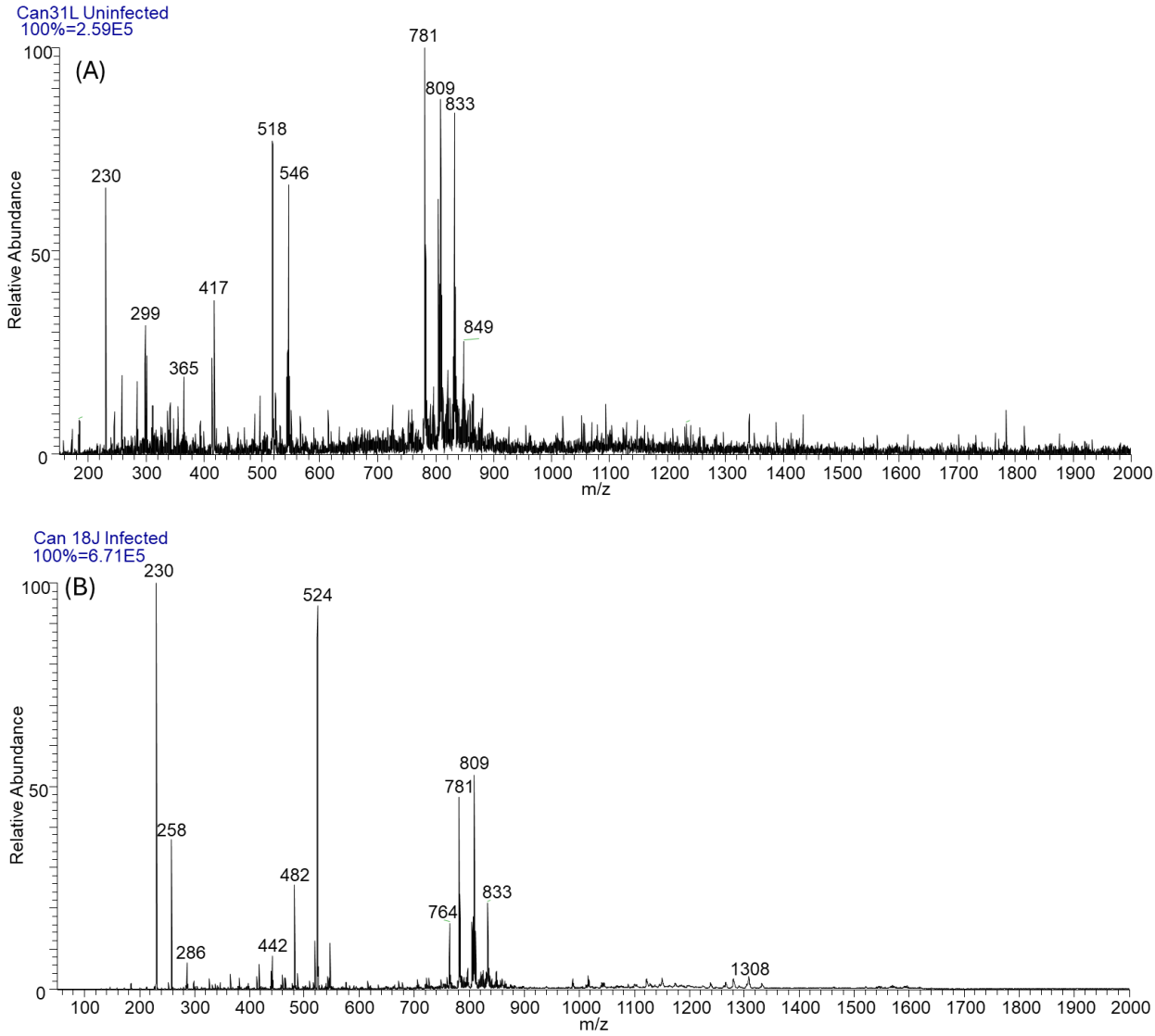

Figure S7. Positive-ion mode flow-through pinhole paper spray mass spectra recorded from (**A**) uninfected and (**B**) infected dried canine serum samples.

6. Statistical Analysis showing VIP scores for top 15 diagnostic metabolites

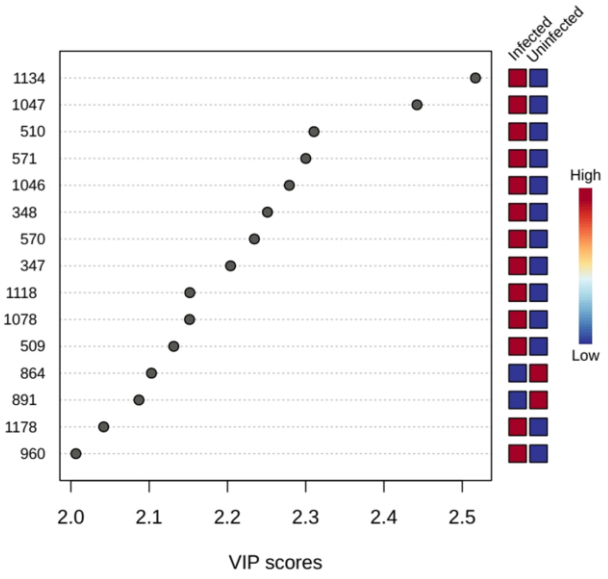

Figure S8. VIP scores showing relative intensities of the top 15 ions in infected versus uninfected samples. Of these, 13 out of the 15 species are upregulated in the infected samples.

7. Antibody Titer of Clinical Samples

Table S1. Sample analyzed in Day 1 (positive and negative), described by sample ID and antibody titer as determined by immunofluorescence assay (IFA).

Day 1		
#	Sample ID	IFA Antibody Titer
Positive		
1	C1B	1/320
2	C2M	1/320
3	C3J	1/320
4	C4T	1/160
5	C5P	1/320
Negative		
6	C36B	-
7	C37L	-
8	C38R	-
9	C39M	-
10	C40L	-

Table S2. Sample analyzed in Day 2 (positive and negative), described by sample ID and antibody titer as determined by immunofluorescence assay (IFA).

Day 2		
#	Sample ID	Antibody Titer based on Immunofluorescence Assay
Positive		
1	CAN9L	1/640
2	CAN12N	1/640
3	CAN16B	1/640
4	CAN18J	1/640
5	CAN20A	1/640
Negative		
6	CAN31L	-
7	CAN32T	-
8	CAN33T	-
9	CAN34B	-
10	CAN35G	-