APPENDIX B - Racing Medication Testing Consortium Unknown Sample Protocol

821 CORPORATE DRIVE · LEXINGTON, KY 40503 · PHONE: 859-224-2844 · FAX: 859-296-3033 · WWW.RMTCNET.COM

Substance Identification Form – Unknown Compounds or Ingredients

Laboratory		**Contact Info:**	
Sample Origin		**Date Submitted**	
Generic Name (if applicable)		**Trade Name**	
Manufacturer/ Compounder		**Lot Number (if applicable)**	

Active Label Ingredients (if appropriate):

Label Photograph:

Container Photograph:

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Testing Performed (please follow Protocol for Identification of Unknown Components or Ingredients):

Fit for Purpose Test	Result	Comment
Screening Test		

Comments:

Please attach a copy of the laboratory report and any associated data to this document for transmittal to RMTC.

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RMTC
Racing Medication & Testing Consortium

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Protocol for Identification of Unknown Components or Ingredients

Purpose:

This protocol is to be used to identify components or ingredients in an unknown substance or an unlabeled or inadequately labeled product. The concentration(s) of identified components or ingredients may be determined after obtaining written authorization from the Executive Director of the RMTC.

Procedure:

1. Complete the attached document regarding the substance or product.

2. Perform a fit for purpose screening test to determine whether regularly tested substances are present in the substances or product.

3. Confirm the identity of each substance detected in the screening test. If additional, unidentified substances remain or if no substances are identified, proceed to step 4.

4. Prepare an appropriate dilution of the substance or product (1:100 or 1:1000 is typical for liquid dose forms) and infuse into an LC-MS system. Monitor in positive and negative ion modes to determine whether ionizable substances are present. Optimize conditions to detect ionizable substances and perform high resolution exact mass LC-MS analysis of the diluted substance or product. Determine the number of components and the exact mass of each component. Perform mass spectral analysis of each component to identify characteristic fragments of the molecular or pseudomolecular ion. Search mass spectral databases and scientific literature for clues to identities of components. If preliminary identifications are made, obtain standards and compare mass spectral and chromatographic data obtained from the unknown substance and the standard to confirm identity of one or more components. If searches of mass spectral databases do not lead to the identification of the unknown substance(s), it will be necessary to attempt to interpret the mass spectral data or to pursue other methods of analysis such as gas chromatography-mass spectrometry (GC-MS). Computer assisted methods for deducing structures of unknown substances are available and may result in compound identifications if the libraries do not contain spectra for those substances. These methods for deducing structure of an unknown substance derive the structure of the unknown through similarities in spectra between the unknown and library spectra and substructures identified from fragmentations.

5. If the components of the mixture cannot be identified by LC-MS methods, perform GC-MS analysis of appropriate extracts of dilutions of the unknown substance or product. Perform

GC-MS analysis without and with derivatization to enhance volatilization of analytes and to improve chromatographic properties. Search electron impact ionization (70 eV) mass spectra against mass spectral libraries such as NIST 11 Mass Spectral Library and Wiley Registry™ of Mass Spectral Data, If matches with library spectra are obtained, reanalyze extract and authentic standards of the analytes from the library search to confirm identities.

6. If the components of the mixture cannot be identified by GC-MS methods, other methods of analysis shall be investigated. Sample preparation methods may need to be developed in order to isolate the unknown substance from solvents and excipients in order to analyze the substance by other methods such as combustion analysis, nuclear magnetic resonance spectrometry, and other methods. Written authorization to pursue these methods shall be obtained from the Executive Director of the RMC before proceeding.