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Written by:	WADA LabEG	Approved by:	WADA LabEG*
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*The approval by the WADA Executive Committee is applicable only to Technical Letters issued after November 2019.

ANALYSIS AND REPORTING OF TRIMETAZIDINE FINDINGS

The *World Anti-Doping Agency* wishes to draw the attention of the Laboratories to the following remarks and instructions on the analysis and reporting of **trimetazidine (TMZ)**.

The detection of TMZ [1-(2,3,4-trimethoxybenzyl)piperazine] in urine may result from the metabolism of **lomerizine** [1-[bis(4-fluorophenyl)methyl-4-(2,3,4-trimethoxybenzyl)piperazine], a permitted drug used for the treatment of migraine.^{1,2,3}

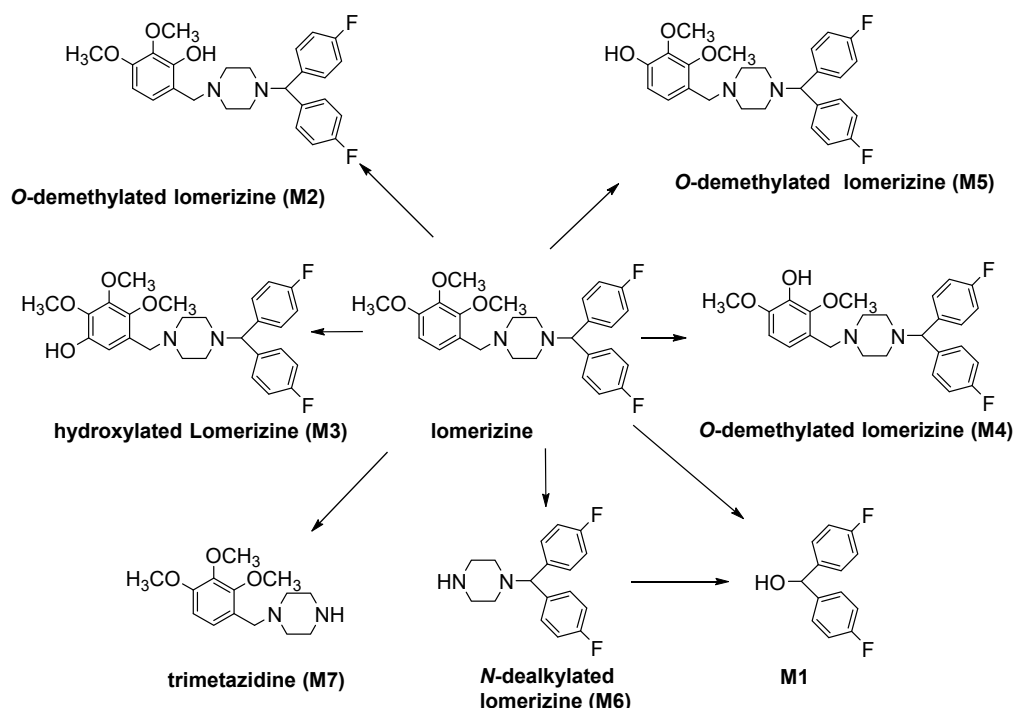


Figure 1. Proposed metabolic pathway of lomerizine (adapted from Okano *et al*³).

Note: Lomerizine (the parent compound), which has a short half-life, can be found in urine at concentrations much lower than that of TMZ (minor *Metabolite*), in a ratio of 1/20 or less. However, the concentration of the lomerizine M6-*Metabolite* is usually higher than that of TMZ.

¹ Awata N, Kawashima T and Sakai T. "Metabolism of lomerizine hydrochloride in humans" *Jpn Pharmacol Ther* **22**: 173-183, 1994.

² Sigmund G *et al*. "Doping control analysis of trimetazidine and characterization of major metabolites using mass spectrometric approaches". *Drug Test Anal.* **6**(11-12):1197-205, 2014.

³ Okano M, Thevis M, Sato M and Kageyama S. "Analytical Detection of trimetazidine produced by metabolic conversion of lomerizine in doping control analysis". *Drug Test Anal.* **8**(8):869-74, 2016.

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Therefore, when detecting TMZ in urine, Laboratories shall:

1. Test for the presence of the non-prohibited lomerizine AND its specific *Metabolite(s)* [at least the N-dealkylated M6 *Metabolite*, 1-Bis-(4-fluorophenyl)-methylpiperazine]⁴ using e.g. LC-MS/MS targeting the diagnostic precursor/product ion pairs shown below, which are obtained from the protonated molecules of TMZ, lomerizine and lomerizine M6-*Metabolite*;

Transition (<i>m/z</i>) ^{3, 5}		
Trimetazidine	Lomerizine	Lomerizine M6- <i>Metabolite</i>
267>181	469>181	289>203
267>166	469>203	289>183
267>136	469>166	

Note: The Limit of Detection (LOD) of the Tests Method(s) used for the detection of lomerizine and its M6 *Metabolite* should be lower than the corresponding LOD for TMZ.

2. Report the result as a Negative Finding if it is considered that the presence of TMZ in the *Sample* could have resulted from the permitted administration of lomerizine;
3. When reporting an *Adverse Analytical Finding* for TMZ, include a comment in the Test Report specifying "Neither lomerizine nor its *Metabolite(s)* (at least M6) were detected in the *Sample*".

Should you have any further questions, please do not hesitate to contact the WADA Science Department.

⁴ The reference standards for lomerizine and its M6 *Metabolite* can be purchased from Sigma-Aldrich (L6295 and 552402, respectively) <https://www.sigmaaldrich.com/catalog/product/aldrich/552402?lang=en®ion=CA>

⁵ Ren Y *et al.* "Determination of lomerizine in human plasma by liquid chromatography/tandem mass spectrometry and its application to a pharmacokinetic study". *J Chromatogr B Analyt Technol Biomed Life Sci.* **947-948**: 96-102, 2014.