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**Report to BioSpectra Inc. for the Analysis of Six Samples of
TRIS, (tromethamine) all GMP products for the Presence of
Four Nitrosamines.**

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<http://www.kinentia.com>

<http://www.kcs-online.com>

Introduction

At the request of BioSpectra Inc. (BSI) Kinentia Biosciences (Kinentia) has conducted testing on six lots of tris base (Tris) a.k.a. Tromethamine or Trisamine, applicable for all GMP excipient codes manufactured by BSI for the presence of the following four nitrosamines:

1. *N,N*-Dimethylnitrosamine (NMDA)
2. *N,N*-Diethylnitrosamine (NDEA)
3. *N*-isopropyl-*N*-Ethylnitrosamine (NPEA)
4. *N*-Methyl, *N*-Phenylnitrosamine (NMPA)

Results

The testing protocol followed that fluorescent tagging methodology recently described by Kinentia in its report titled *Final Report to BioSpectra Inc. for Development of an Analytical Method for the Detection of Various Nitrosamines* dated March 31, 2021. The results were observed following this testing protocol are delineated in Table 1 below.

Nitrosamines **2**, **3** and **4** were undetected using the fluorescence tagging protocol previously described using 5-(dimethylamino)naphthalene-1-sulfonyl chloride (dansyl chloride). Due to the presence of a co-eluting impurity the absolute quantitation of nitrosamine **1** was not possible. However, the use of an authentic sample of the dansylamide derived from **1** as a reference standard allowed for the relative quantitation of **1** by comparisons of HPLC AUC values. Using this methodology, it could be determined that less than 96 µg / mL was present in all samples submitted.

Presence of N-Nitrosodiisopropylamine and N-Nitroso-4-aminobutyric acid.

Whereas analysis for the presence of *N*-Nitrosodiisopropylamine and *N*-Nitroso-4-aminobutyric acid was not conducted, analysis for the manufacturing process for trisamine reveals that the possibility for the presence of these two nitrosamines is very limited. Since no reagents or solvents are utilized in the manufacturing process for trisamine that could generate these nitrosamines, their presence in the final material is very unlikely.

Table 1

Lot Number	Testing Result
TRIS-3200-01-0321	NMDA – <96ng / mL NDEA – ND NPEA – ND NMPA - ND
TRIS-3200-02-0321	NMDA - < 96ng / mL NDEA – ND NPEA – ND NMPA - ND
TRIS-3200-03-0321	NMDA - < 96ng / mL NDEA – ND NPEA – ND NMPA - ND
TRIS-3200-04-0321	NMDA - < 96ng / mL NDEA – ND NPEA – ND NMPA - ND

ND = Not detected

Please note, TRIS-3200 is BSI's internal intermediate bulk FG product code for all GMP excipient Tris products including but not limited to Finished Good GMP Product Codes: TRIS-3220; TRIS-3251, TRIS-3254; TRIS-3255