March 2024

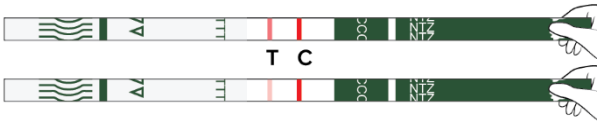
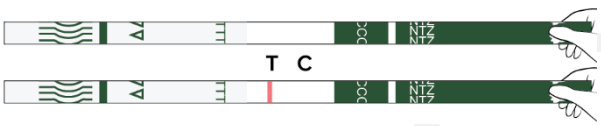
BTNX Rapid Response™ Nitazene Test Strip 2000 ng/mL

Introduction

Starting from 2019, the prevalence of the NPS opioid subclass known as 2-benzylbenzimidazoles or 'nitazenes' began to rise, causing concern due to suspected high potency. Originally discovered in the late 1950s by the pharmaceutical company CIBA, this potent opioid subclass entered the recreational drug market following the scheduling of fentanyl-class drugs in 2018, marked by the emergence of isotonitazene. Isotonitazene quickly gained popularity, contributing to numerous fatalities in the U.S. alone. It dominated the U.S. novel synthetic opioid market for approximately a year, eventually leading to its classification under Schedule I control by the U.S. Drug Enforcement Administration (DEA).

This class of substances contains a benzimidazole ring with an ethylamine at its 1-position and a benzyl group at its 2-position. Small structural modifications to this scaffold can produce a series of analogous substances. Nitazenes are ultra-high potency synthetic opioids, Isotonitazene and Protonitazene are up to 8 times stronger than fentanyl, while etonitazepyne and protonitazepyne surpass fentanyl's potency by a staggering 20 times.

The Rapid Response™ Nitazene Test Strip (NTZ-18S26) from BTNX is specifically designed for the swift screening of nitazenes in liquid/powder samples. This test incorporates an antibody that selectively identifies nitazenes. Similar to other harm reduction test strips, it operates on the competitive binding principle. If nitazene is present in the sample at a concentration surpassing the designated cut-off, a singular-colored line will appear in the test.

Positive - Nitazene Detected Only one colored line appears in the control region (C). No apparent colored line appears in the test region (T).	
Negative – Nitazene Could Not be Detected Two colored lines appear on the membrane. One line appears in the control region (C) and another line appears in the test region (T).	
Invalid Control line fails to appear. Results from any test which has not produced a control line at the specified read time must be discarded. Please review the procedure and repeat with a new test. If the problem persists, discontinue using the kit immediately and contact your local distributor.	

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Test Performance

Analytical Sensitivity

The following compounds were spiked into water, respectively, to examine the ability of the test strip for detecting nitazene analogues. The cut-off listed in the table represents the concentration at which a substance will begin to show a mix of positive and negative results.

Nitazene analogues that can be detected below 100 µg/mL	
Compounds	**Cut off**
Isotonitazene	2000 ng/mL
Protonitazene	4500 ng/mL
N-pyrrolidino Etonitazene	1300 ng/mL
Etonitazene	2000 ng/mL

Nitazene analogues that can NOT be detected below 100 µg/mL	
Compounds	**Cut off**
Metodesnitazene (Cerilliant®)	> 100 µg/mL
Etodesnitazene (Cerilliant®)	> 100 µg/mL

Analytical Specificity

The following compounds were spiked into water, respectively, to examine possible cross-reactivity. No false positives were observed at the concentrations listed in the table.

Compounds	Concentration	Compounds	Concentration
Xylazine	5 mg/mL	Acetaminophen	5 mg/mL
MDMA	5 mg/mL	Heroin	1 mg/mL
Methamphetamine	5 mg/mL	Diphenhydramine	1 mg/mL
Quinine	5 mg/mL	Methadone	0.1 mg/mL
Quinidine	5 mg/mL	Fentanyl	0.1 mg/mL
Lidocaine	5 mg/mL	Cocaine	0.1 mg/mL
Levamisole	5 mg/mL	Ketamine	0.1 mg/mL
Caffeine	5 mg/mL	Tramadol	0.1 mg/mL

Third Party Evaluations

Center for Forensic Science Research & Education (CFSRE)

The Center for Forensic Science Research & Education (CFSRE) conducted an independent evaluation of the Rapid Response™ Nitazene Test Strip. To test its effectiveness in detecting nitazene analogues, various compounds were introduced into water at a concentration of 3000 ng/mL. The outcomes are detailed in the following tables

Nitazene analogues detected at 3000 ng/mL	
4'-Hydroxy Nitazene	5-Aminoisotonitazene
5-Methyl Etodesnitazene	Clonitazene
Ethyleneoxynitazene	Etodesnitazene
Etonitazene	Protonitazene
Isotonitazene	Flunitazene
Nitazene analogues detected at 3000 ng/mL cont.	
Methylenedioxynitazene	Menitazene

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Metonitazene	Metodesnitazene (Cayman)
N-Desethyl Isotonitazene	Nitazene
N-Desethyl Protonitazene	N-Desethyl Metonitazene
N-Pyrrolidino 4'-Hydroxy Nitazene	N-Piperidinyl 4'-Hydroxy Nitazene
N-Pyrrolidino Isotonitazene	N-Pyrrolidino Etonitazene
N-Pyrrolidino Metonitazene	N-Pyrrolidino Protonitazene

Nitazene analogues NOT detected at 3000 ng/mL	
Butonitazene	Isotodesnitazene
Protodesnitazene	N-Pyrrolidino Etodesnitazene
N-Pyrrolidino Metodesnitazene	

Chicago Recovery Alliance

In a separate assessment, the Chicago Recovery Alliance analyzed the strip's performance with real-world street drug samples. With a dilution factor of 10 mg per 1 mL (one scoop in 1 mL of water), the strip achieved 100% sensitivity and specificity. When diluted to 10 mg per 5 mL (one scoop in 5 mL of water), sensitivity was observed at 89%, and specificity remained at 100%. The investigation continues, with the preliminary findings shown in the table below.

Sample Code	10mg / 1mL (POS)	10mg / 5mL (POS)	Contains Nitazene	Contains Caffeine	Fentanyl : Nitazene	Fentanyl : Caffeine	DrugsData Link	Nitazene Analogues
Y4 319	TRUE	FALSE	TRUE	FALSE	90 : 1	-	https://www.drugsdata.org/view.php?id=16824	Metonitazene
Y4 238	TRUE	TRUE	TRUE	FALSE	50 : 1	-	https://drugsdata.org/view.php?id=15549	Metonitazene
Y4 243	TRUE	TRUE	TRUE	FALSE	30 : 1	-	https://drugsdata.org/view.php?id=15846	Metonitazene
Y4 317	TRUE	TRUE	TRUE	FALSE	11 : 1	-	https://www.drugsdata.org/view.php?id=16818	Metonitazene
Y4 245	TRUE	TRUE	TRUE	FALSE	8 : 1	-	https://drugsdata.org/view.php?id=15843	Metonitazene
Y4 250	TRUE	TRUE	TRUE	FALSE	8 : 1	-	https://drugsdata.org/view.php?id=15848	Metonitazene
Y4 348	TRUE	TRUE	TRUE	FALSE	1 : 6	-	https://www.drugsdata.org/view.php?id=16817	Metonitazene/Protonitazene
Y4 315	TRUE	TRUE	TRUE	FALSE	4 : 10	-	https://www.drugsdata.org/view.php?id=16812	N-Pyrrolidino Protonitazene
Y4 332	TRUE	TRUE	TRUE	FALSE			Above 5% in the mixture, no GC/MS	Protonitazene
Y4 480	FALSE	FALSE	FALSE	TRUE	-	4 : 5	https://www.drugsdata.org/view.php?id=16918	
Y4 292	FALSE	FALSE	FALSE	TRUE	-	2 : 1	https://drugsdata.org/view.php?id=15910	
Y4 291	FALSE	FALSE	FALSE	TRUE	-	3 : 1	https://drugsdata.org/view.php?id=15914	