Specialist in chemical analysis

Expert in Nitrosamine analysis

Introduction

Brightlabs is the laboratory for all your analytical questions. Located in the south of The Netherlands, close to the German and Belgium border, it is our mission to support our customers in their pharmaceutical development projects as well as pharmaceutical QC testing.

In finding the correct solution we work in close contact with our customers by Co-Creation, to achieve your end goals in the most efficient manner to maximize your success.

Of course Brightlabs has a GMP permit and opiate permits to work on drug substances and drug products.



Services

Brightlabs offers a complete service ranging from physical, chemical and microbiological quality control testing of pharmaceutical products, from raw materials to finished dosage forms.

We offer a wide range of support at every stage of your drug development. Our capabilities include; method development and validation, elemental impurity, microbiological testing, stability studies, cleaning validations, quality control testing and much much more.

Nitrosamines

In response to nitrosamines present in pharmaceutical products, the European Medical Agency (EMA) and the US Food and Drug Administration (US FDA) have published requirements and limits related to nitrosamine contaminants.

Therefore Brightlabs has developed and validated multiple methods (GC-MS/MS & LC-MS/MS) to determine low level concentrations of Nitrosamines.

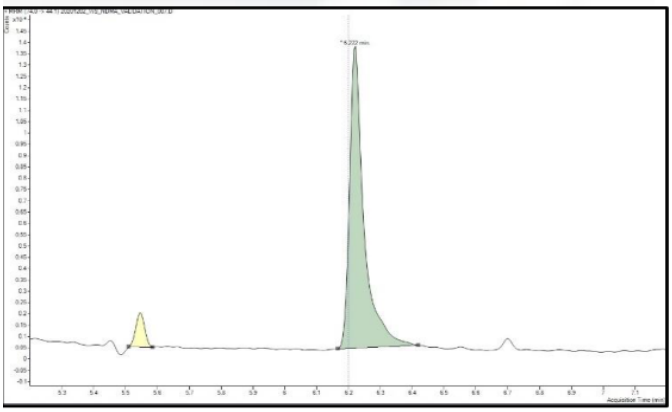
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NDMA & Nitrosamines Screen

NDMA

One of the most common nitrosamine is probably NDMA (N-Nitrododimethylamine), which has been found in 2019 in some over de counter drug products based on Ranitidine. But also in some products used in the treatment of type 2 diabetes.

Brightlabs has developed a method for as well drug substances as drug products to measure NDMA in low levels (lower than 1 ppb, depending on product). This method can easily be adapted to different drug substances and products as part of a risk assessment or for the release of products as part of your QC check.



Chromatogram of NDMA at 1,25 ppb

Nitrosamines in the Brightlabs Screen		
Afkorting	**Nitrosamine**	**CAS**
NDMA	N-Nitrosodimethylamine	62-75-9
NDEA	N-Nitrosodiethylamine	55-18-5
NEIPA	N-Nitrosoethylisopropylamine	16339-04-1
NDIPA	N-Nitrosodiisopropylamine	601-77-4
NMBA	4-(methyl)(nitroso)amino)butanoic acid	61445-55-4
MeNP	1-Methyl-4-nitrosopiperazine	16399-07-4
NDBA	N-Nitrosodibutylamine	924-16-3
NMPA	N-Nitroso-N-methylaniline	614-00-6
NMOR	N-nitrosomorpholine	59-89-2
NMEA	N-nitrosomethylethylamine	10595-95-6
NDPA	N-nitroso-di-n-propylamine	621-64-7
NPYR	N-nitrosopyrollidine	930-55-2
NPIP	N-nitrosopiperidine	100-75-4
NIPEA	N-Nitrosoisopropylethylamine	16339-04-1

Nitrosamines Screen

Brightlabs has multiple in-house developed and validated methods for the determination of low-level Nitrosamines screen using LC-MS/MS and GC-MS/MS. The most important Nitrosamines as listed by the EMA are present in these methods, and can be measured in very low levels, even as low as < 1 ppb depending on the product and nitrosamine. The generic methods can be easily adapted to different drug substances. Of course, this generic method must be validated for work under GMP for each product, for which Brightlabs has a simple but effective protocol.

GC-MS/MS

Our GC method is using a Liquid Liquid Extraction (LLE) sample preparation. The extraction has several advantages like a high capacity of the extractant and high selectivity of separation. Of course for each API, Drug product or other matrix a Suitability test should be performed to test the efficiency of the extraction and the influence on the analysis method.

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GC-MS/MS method performance

GC-MS/MS

For the analyses a GC-MS/MS method is being used, with optimized mass settings for all the different Nitrosamines. See an overview in the table below.

Very low LOQ for each individual nitrosamine can be achieved using GC-MS/MS analysis (as low as 0.008 ppb) in pure solvent, as shown in the table below. During a MST the influence of the matrix is being tested, and the LOQ in the sample is calculated.

An example of a GC-MS/MS chromatogram is given on the next page.

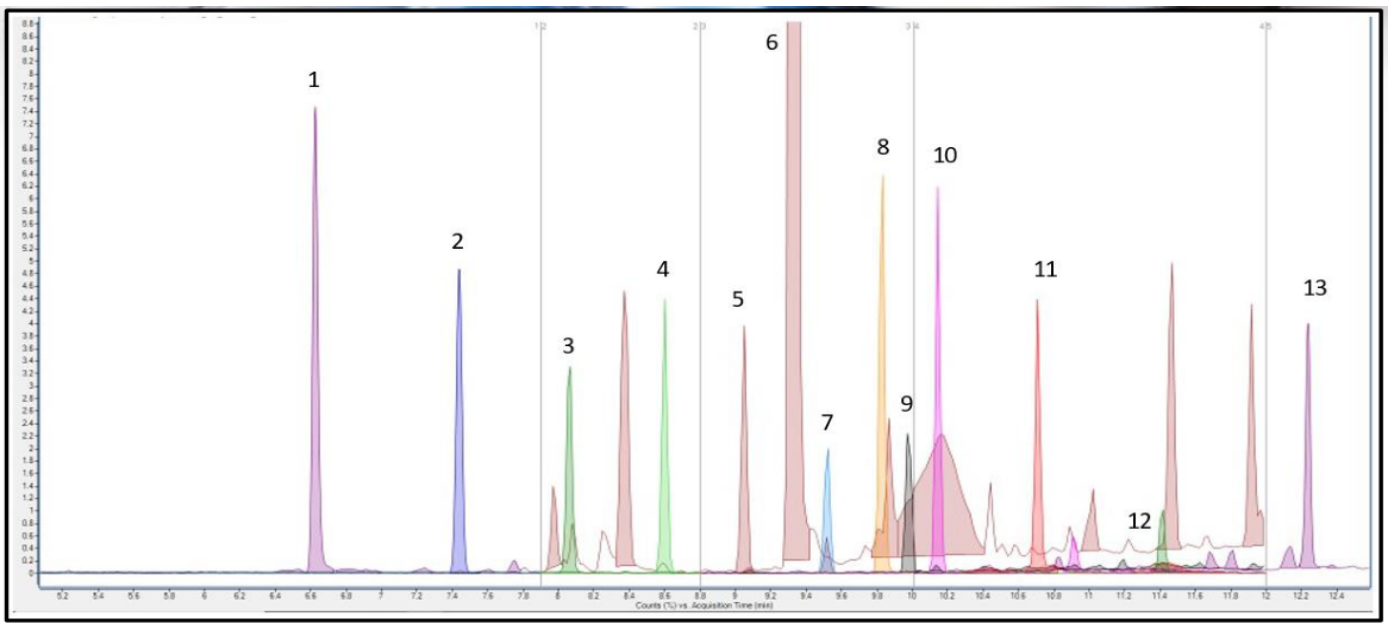
Overview MRM settings Nitrosamines

Nitrosamine	MRM transition	Collision Energy
NDMA	74.0 -> 44.1	5
NMEA	88.0 -> 71.0	3
NDEA	102.0 -> 85.0	3
NEIPA	116.0 -> 99.0	10
NDIPA	130.0 -> 88.0	2
NMPA	106.0 -> 77.0	10
NDPA	101.0 -> 70.0	2
NMOR	116.0 -> 86.0	2
NPYR	100.0 -> 55.0	7
NPIP	114.0 -> 84.0	8
NDBA	116.0 -> 99.0	2
NMBA	130.0 -> 98.0	1
NPHA	168.1 -> 139.0	60

Calculated LOD/LOQ

Nitrosamines	R2	LOD (ppb)	LOQ (ppb)	Lineair meetbereik (ppb)
NDMA	0.9994	0.003	0.008	2.5-7.5
NMEA	0.9994	0.002	0.008	2.5-7.5
NDEA	0.9989	0.004	0.015	2.5-7.5
NEIPA	0.9997	0.002	0.006	2.5-7.5
NDIPA	0.9994	0.001	0.004	2.5-7.5
NMPA	0.9947	0.009	0.028	25-75
NDPA	0.9960	0.006	0.019	2.5-7.5
NMOR	0.9972	0.006	0.020	2.5-7.5
NPYR	0.9981	0.043	0.143	2.5-7.5
NPIP	0.9974	0.009	0.031	2.5-7.5
NDBA	0.9902	0.009	0.031	2.5-7.5
NMBA	0.9956	0.053	0.178	25-75
NPHA	0.9997	0.011	0.038	2.5-7.5

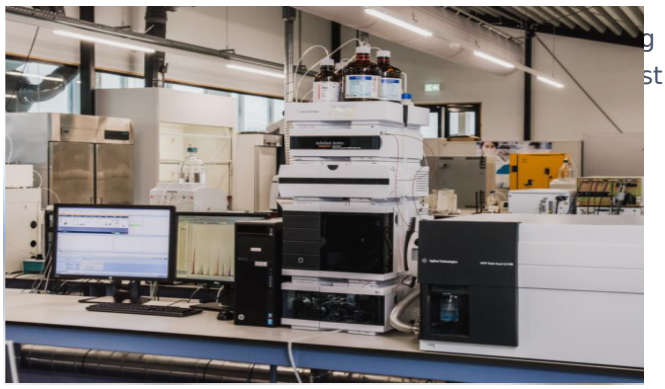
Screening & LC-MS/MS



GC-MS/MS Chromatogram of screening multiple Nitrosamines

LC-MS/MS

For the analyses also UHPLC-(APCI)-MS/MS methods are being used, with the optimized mass settings for all the different Nitrosamines.



Overview MRM settings Nitrosamines

Naam	MRM transition	Collision Energy
NMBA	100.20 > 58.05	-25
MENP	130.00 > 58.10	-22
NDMA	75.20 > 58.10	-18
NMOR	117.00 > 87.10	-15
NMEA	89.20 > 61.10	-16
NPYR	101.00 > 55.10	-17
NDEA	103.00 > 75.10	-15
NEIPA	117.00 > 75.05	-12
NPIP	115.00 > 69.10	-17
NDIPA	131.00 > 89.10	-13
NDPA	131.00 > 89.10	-13
NMPA	137.30 > 66.05	-22
NDBA	159.20 > 57.15	-15
NDPHA	170.00 > 93.10	-26

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UHPLC-MS/MS results

Calculated LOD/LOQ

Nitrosamines	R2	LOD	LOQ	Lineair range (ppb)
NMPA	0,9986	0,8	2,8	3-25
NMOR	0,9979	4,3	14,4	15-25
NMEA	0,9997	0,7	2,2	3-25
NDEA	0,9986	0,7	2.1	3-25
NEIPA	0,9994	0,5	1,6	3-25
NPIP	0,9982	0,5	1,7	3-25
NDIPA	0,9997	0,7	2,4	3-25
NDPA	0,9985	0,6	1,9	3-25
NDBA	0,9997	0,5	1,7	3-25
NDPHA	0,9991	0,6	2,1	3-25
NPYR	0,9984	2,9	9,6	10-100
NDMA	0,9978	6,0	20,1	20-100
NMBA	0,9966	2,1	6,9	10-100
MENP	0,9998	0,3	0,8	3-25

UHPLC-MS/MS

Using APCI-MS/MS still very low LOQ's can be reached, with still an acceptable lineair range.

Depending on the requested nitrosamines, a combination of UHPLC-MS/MS and GC-MS/MS methods can be used.

All methods can easily be extended with product specific nitrosamines. Brightlabs team of specialist are well trained and experienced to perform a method development and validation specific on your request.

Contact

Do you have any questions regarding the nitrosamine or other analysis? Do not hesitate to contact us.

Interested what Brightlabs can do for you? Please contact us by mail or telephone.

Contact@Brightlabs.nl



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