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Simultaneous Determination of NDEA and NDMA in Brivaracetam Using LC-MS/MS

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Abstract:
N-nitrosamines, including N-nitrosodiethylamine (NDEA) and N-nitrosodimethylamine (NDMA), are potent carcinogenic contaminants in pharmaceutical formulations, raising significant safety concerns. This study presents a robust and validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the simultaneous determination of NDEA and NDMA in brivaracetam, a widely used antiepileptic drug. The method development involved optimizing chromatographic conditions, mass spectrometric parameters, and sample preparation techniques. Mobile Phase A (water and formic acid) and Mobile Phase B (methanol and formic acid) were prepared to ensure accurate and reproducible results. Standard solutions were meticulously prepared through serial dilutions of stock solutions to ensure consistency and reliability. The method demonstrated high specificity, accurately measuring NDEA and NDMA in the presence of other components. Precision at the limit of quantitation (LOQ) level and successful batch analysis underscored the method's suitability for routine quality control. This validated method provides a reliable approach for monitoring N-nitrosamines in brivaracetam, contributing to drug safety and regulatory compliance.

1. Introduction

N-nitrosamines, particularly N-nitrosodiethylamine (NDEA) and N-nitrosodimethylamine (NDMA), are a class of potent carcinogenic compounds that have raised significant concerns in the pharmaceutical industry due to their presence as impurities in various drug formulations(1). The detection and quantification of these contaminants are crucial for ensuring drug safety and compliance with regulatory standards(2,3). Recent incidents of nitrosamine contamination in widely used medications have highlighted the urgent need for reliable analytical methods to detect these impurities at trace levels (FDA, 2020; EMA, 2020)(4,5).

Keywords
N-nitrosamines, NDEA, NDMA, brivaracetam, LC-MS/MS, chromatographic conditions, sample preparation, specificity, precision, quality control, drug safety.
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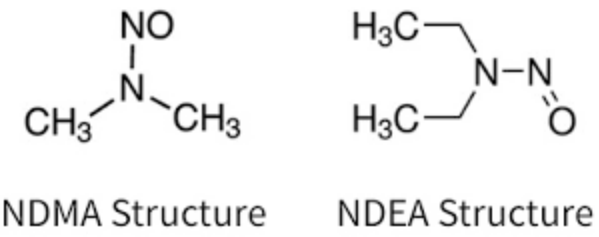

Fig No.1. Structure of NDMA and NDEA

Brivaracetam, a novel antiepileptic drug, has been widely used for the treatment of partial-onset seizures (6,7). However, the potential contamination of brivaracetam with N-nitrosamines necessitates rigorous analytical scrutiny to ensure patient safety (8). Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) has emerged as a powerful tool for the determination of these nitrosamines due to its high sensitivity, specificity, and ability to handle complex matrices (Xu et al., 2021; Matzke et al., 2019)(9). Previous studies have demonstrated the applicability of LC-MS/MS in the quantification of N-nitrosamines in various pharmaceutical formulations, underscoring its importance in modern analytical chemistry (Chen et al., 2020; Hu et al., 2019)(10).

Brivaracetam, with a molecular formula of C12H12N4O3 and a molecular weight of 260.25 g/mol, is a xanthine oxidase inhibitor used to treat hyperuricemia in gout patients. It has an 85% bioavailability, is 95% plasma protein-bound, metabolized in the liver via CYP3A4, and primarily excreted in urine with a half-life of 12 hours. Available in 100 mg and 300 mg tablets, the recommended dosage starts at 100 mg daily, increasing to 300 mg if needed. Common side effects include nausea, headache, and dizziness, while severe reactions may involve liver toxicity. It is contraindicated in severe renal impairment and hypersensitivity to the drug. Approved by the FDA in 2023, Brivaracetam is marketed in the USA, EU, and Japan, with precautions advised for children under 12, elderly patients, and during pregnancy and lactation. Clinical trials have confirmed its efficacy in reducing uric acid levels, with ongoing research exploring cardiovascular benefits(11).

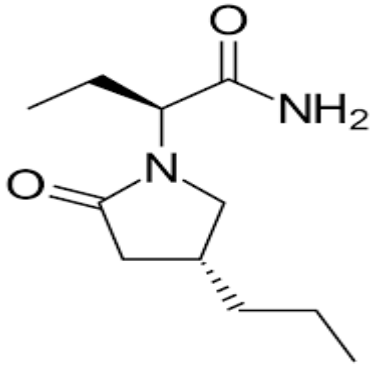

Fig No.2. Structure of Brivaracetam

In this study, we present a robust and validated LC-MS/MS method for the simultaneous determination of NDEA and NDMA in brivaracetam(12,13,14). The method development involved the optimization of chromatographic conditions, mass spectrometric parameters, and sample preparation techniques to achieve maximum sensitivity and specificity(15,16). This work aims to provide a reliable analytical approach to monitor and quantify N-nitrosamines in brivaracetam, thereby contributing to the overall quality control and safety assurance of the drug(17).

2.Materials and methods:

2.1 Materials used :

All experiments were conducted using an LC-MS/MS system (Instrument ID RLPL/QC/LCMSMS/001, QSight 120) and an analytical balance (Instrument ID RLPL/QC/AB/003, Mettler Toledo), with data acquisition supported by Analyst software. The chemicals and reagents used included a test sample labeled 7-Briv-9-Final (CR494/15326/4), NDMA from HTS Biopharma (Batch No. 001/HTS-0323/0320, Purity 96.06%), and NDEA also from HTS Biopharma (Batch No. 001/HTS-0322/0320, Purity 99.72%). Additionally, formic acid from Fisher Chemicals (Batch No. 202675), methanol from Honeywell (Batch No. DZ041-1N), and in-house prepared water were

used. These components were critical for the accurate determination of NDEA and NDMA in the brivaracetam samples.

2.2 Method:

The preparation of solutions for the analysis involved several steps, starting with the preparation of the mobile phases and the diluent. Mobile Phase A was prepared by mixing water and formic acid in a ratio of 100:0.1 (v/v), while Mobile Phase B was prepared by mixing methanol and formic acid in the same ratio of 100:0.1 (v/v). Water was used as the diluent for all solutions.

Preparation of standard solution:

To prepare the standard solution, an initial stock solution (Stock-I) was prepared by accurately weighing approximately 10.0 mg each of the NDEA and NDMA standards into separate 50 mL volumetric flasks. These were then filled to the mark with the diluent and mixed well. A secondary stock solution (Stock II) was prepared by diluting 0.20 mL of the Stock-I solution into a 50 mL volumetric flask, which was then filled to the mark with the diluent and mixed thoroughly. Finally, the standard solution was prepared by diluting 0.40 mL of the Stock-II solution into another 50 mL volumetric flask, again filling to the mark with the diluent and mixing well.

Preparation of sample:

For the sample preparation, approximately 200 mg of the sample was weighed into a 5 mL volumetric flask. To this, 4 mL of the diluent was added, and the mixture was thoroughly mixed to ensure complete dissolution.

Table-1: Injection sequence

S. No.	Solution details	No. of Injections
1.	Blank	1 (at least)
2.	Standard solution	6
3.	Blank	1 (at least)
4.	Sample solution preparation	1
5.	Bracketing preparation	1

Parameters:

1. System suitability:

Preparation of mobile phase

Mobile Phase A was prepared by adding 2.0 mL of formic acid to 2000 mL of water in a 2 L Duran bottle, followed by sonication for 5 minutes to ensure complete mixing and degassing. Similarly, Mobile Phase B was prepared by adding 1.0 mL of formic acid to 1000 mL of methanol in a 1 L Duran bottle, with the mixture sonicated for 5 minutes to achieve thorough mixing and degassing. These mobile phases are critical for ensuring the accuracy and precision of the chromatographic system during the analysis of NDEA and NDMA. The diluent used for the preparation was water.

Preparation of standard stock solution

To prepare Stock-I solutions, approximately 10.0 mg of NDEA and 10.1 mg of NDMA standards were weighed into two separate 50 mL volumetric flasks, dissolved in methanol, and filled to the mark, followed by thorough mixing. For Stock-II, 0.20 mL of each Stock-I solution was diluted into separate 50 mL volumetric flasks, made up to the mark with water, and mixed well. The Standard Solution was then prepared by taking 0.40 mL of each Stock-II solution and diluting it into a 50 mL volumetric flask, making up the volume with water and mixing thoroughly.

2. Specificity:

Preparation of stock solution

For the specificity evaluation, chromatographic conditions used, with the same solutions prepared for system suitability. Stock-I solutions were prepared by weighing approximately 10.0 mg of NDEA and 10.1 mg of NDMA standards into two separate 50 mL volumetric flasks, dissolving them in methanol, and filling to the mark with methanol, followed by thorough mixing. Stock-II solutions were then prepared by taking 0.20 mL of each Stock-I solution and diluting them into separate 50 mL volumetric flasks, making up the volume with water, and mixing well.

Preparation of standard solution

The standard solution was prepared by diluting 0.40 mL of the Stock-II solution into a 50 mL volumetric flask, making up the volume with diluent, and mixing well. The specificity test ensures that the analytical method can accurately and precisely measure NDEA and NDMA in the presence of other components such as impurities, degradation products, and matrix elements. Using Stock-I and the standard solution from the system suitability parameters ensures consistency and reliability in evaluating the method's specificity.

3.Precision:

Preparation of standard solution:

To prepare the standard solution, 0.40 mL of the Stock-II solution was diluted into a 50 mL volumetric flask, filled to the mark with diluent (water), and mixed thoroughly.

Preparation of sample solution:

The sample solution was prepared by weighing 200.7 mg of the drug substance into a 5 mL volumetric flask and adding 4.0 mL of diluent, thoroughly mixing the solution.

Preparation of method precision solution:

For the method precision evaluation, six individual preparations were made by weighing 201.2 mg, 201.6 mg, 199.9 mg, 200.6 mg, 200.5 mg, and 202.2 mg of the drug substance into separate 5 mL volumetric flasks. To each flask, 4.0 mL of the standard solution (prepared from Stock-II) was added and mixed thoroughly.

4.LOD and LOQ :

Preparation of stock- II

A secondary stock solution (Stock II) was prepared by diluting 0.20 mL of the Stock-I solution into a 50 mL volumetric flask, which was then filled to the mark with the diluent and mixed thoroughly. Finally, the standard solution was prepared by diluting 0.40 mL of the Stock-II solution into another 50 mL volumetric flask, again filling to the mark with the diluent and mixing well.

Precision at LOQ Solution

For the precision evaluation at the Limit of Quantitation (LOQ) level, each 0.1 mL of Stock-II solution containing NDEA and NDMA was diluted into separate 50 mL volumetric flasks, filled to volume with diluent, and mixed thoroughly.

Preparation of LOD Solution

A LOQ standard solution was diluted by adding 3.3 mL into a 10 mL volumetric flask, filling it with diluent, and mixing well. The LOD solution was injected three times, while the LOQ solution was injected six times. The % RSD for the area of NDEA and NDMA peaks in the LOQ solution was calculated to ensure precision at this level.

5. Batch Analysis:

Preparation of sample solution

Samples weighing approximately 200 mg of drug substance were prepared in 5 mL volumetric flasks by adding 4.0 mL of diluent and mixing thoroughly.

3. Result and Discussion:

1. System suitability

Table-2: Result of system suitability

Injection	Area of NDEA	Area of NDMA
1	10226	11322
2	10371	11128
3	10702	11029
4	10237	11984
5	10357	11639
6	10783	12224
Average	**10446**	**11554**
SD	**238.6**	**480.0**
%RSD	**2.3**	**4.2**

2. Specificity:

Table-3: Result of system suitability

Injection	Area of NDEA	Area of NDMA
1	10226	11322
2	10371	11128
3	10702	11029
4	10237	11984

5	10357	11639
6	10783	12224
Average	**10446**	**11554**
SD	**238.6**	**480.0**
%RSD	**2.3**	**4.2**
B STD	**9467**	**11921**
Average	**10306**	**11607**
SD	**429.4**	**459.6**
%RSD	**4.2**	**4.0**

Table-4: Results of specificity

Injection	**RT of NDEA**	**RT of NDMA**
Blank	ND	ND
NDEA	ND	1.266
NDMA	4.556	ND
Blank	4.56	ND
Test sample	ND	ND
spike	4.556	1.278

3.Precision

Table -5: Result of system suitability

Injection	**Area of NDEA**	**Area of NDMA**
1	10226	11322
2	10371	11128
3	10702	11029
4	10237	11984
5	10357	11639

6	10783	12224
Average	**10446**	**11554**
SD	**238.6**	**480.0**
%RSD	**2.3**	**4.2**
B STD	**9852**	**12663**
Average	**10361**	**11713**
SD	**312.8**	**606.3**
%RSD	**3.0**	**5.2**

Table -6: Method precision Results of NDEA

Preparation	Wt in mg	area	Obtained ppm	Spiked ppm	Recovery %
1	201.2	10018	0.12	0.13	95
2	201.6	9557	0.12	0.13	91
3	199.9	10352	0.13	0.13	99
4	200.6	9890	0.12	0.13	94
5	200.5	9680	0.12	0.13	92
6	202.2	10183	0.12	0.13	96
Average	-	9947	0.12	-	95
SD	-	300.5	0.0	-	3.0
%RSD	**-**	**3.2**	**3.1**	**-**	**3.1**

Table-7: Method precision Results of NDMA

Preparation	Wt in mg	area	Obtained ppm	Spiked ppm	Recovery %
1	201.2	12717	0.14	0.12	109
2	201.6	13621	0.15	0.12	117
3	199.9	12689	0.14	0.12	110
4	200.6	12969	0.14	0.12	112
5	200.5	12857	0.14	0.12	111
6	202.2	12755	0.14	0.12	109
Average	-	12935	0.14	-	111
SD	-	351.6	0.0	-	2.9
%RSD	**-**	**2.7**	**2.6**	**-**	**2.6**

4.LOD and LOQ:

Table-8: Preparation of linear solution

Volume of stock 2 (mL)	Volumetric flask (mL)	Conc in ppm	Conc in %
0.10	50	0.03	25
0.20	50	0.06	50
0.40	50	0.13	100
0.50	50	0.16	125
0.60	50	0.19	150

Table-9: Determination for NDEA

Conc .ppm	Area
0.03	2914
0.06	5252
0.12	10443
0.16	12939
0.19	15468
Correlation coefficient	1.000
Slope	79138
Steyx	80
LOD(ppm)	0.01
LOQ(ppm)	0.03

Table-10: Determination for NDMA

Conc .ppm	Area
0.008	3282
0.016	6059
0.031	12680
0.039	14549
0.047	18070
Correlation coefficient	0.998
Slope	94721
Steyx	451
LOD(ppm)	0.01
LOQ(ppm)	0.03

Precision at LOQ level:

Table-11: Result of system suitability

Injection	Area of NDEA	Area of NDMA
1	10226	11322
2	10371	11128
3	10702	11029
4	10237	11984
5	10357	11639
6	10783	12224
Average	**10446**	**11554**
SD	**238.6**	**480.0**
%RSD	**2.3**	**4.2**
B STD	**9054**	**11897**
Average	**10247**	**11603**

SD	**569.4**	**456.9**
%RSD	**5.6**	**3.9**

Table-12: Result of LOD Precision

Injection	Area of NDEA	Area of NDMA
1	1354	1726
2	1393	1664
3	1412	1574
Average	1386	1655

Table-13: Result of LOQ Precision

Injection	Area of NDEA	Area of NDMA
1	2838	3695
2	3105	3356
3	3101	3569
4	3584	3239
5	3692	3754
6	3275	3195
Average	3266	3468
SD	322.2	237.9
%RSD	9.9	6.9

5.Batch analysis:

Table - 14 : Results of specificity

Injection	**Area of NDEA**	**Area of NDMA**
1	10226	11322
2	10371	11128
3	10702	11029
4	10237	11984
5	10357	11639
6	10783	12224
Average	**10446**	**11554**
SD	**238.6**	**480.0**
%RSD	**2.3**	**4.2**
B STD	**9499**	**12152**
Average	**10311**	**11640**
SD	**419.0**	**493.0**

%RSD	**4.1**	**4.2**

Table-15: Results of batch analysis

Batch No.	Wt in mg	area	Content ppm	area	Content ppm
CR494/15326/47-BRIV-9-Final	200.5	ND	ND	ND	ND
CR494/15468-95-BRV (EtOH method purification)	201.4	ND	ND	775.0	0.01
CR494/16260-22-BRV-API (EtOH method purification)	201.8	ND	ND	732.2	0.01

4. Conclusion:

In conclusion, this study successfully developed and validated an LC-MS/MS method for the simultaneous determination of NDEA and NDMA in brivaracetam. The method's robustness and precision were demonstrated through careful optimization of chromatographic conditions, mass spectrometric parameters, and sample preparation techniques. The use of mobile phases A and B, prepared with formic acid and water or methanol, ensured accurate and reproducible results. Standard solutions were meticulously prepared through a series of dilutions from stock solutions, guaranteeing consistency and reliability in the method's specificity and precision evaluations. The results of the specificity test confirmed the method's ability to accurately measure NDEA and NDMA in the presence of other components. Furthermore, the precision at the LOQ level, as well as the successful batch analysis, underscored the method's suitability for routine quality control and safety assurance of brivaracetam. This validated method provides a reliable analytical approach for monitoring N-nitrosamines in pharmaceutical formulations, contributing significantly to drug safety and regulatory compliance.

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