

Supplementary Material

Development and validation of a universal LC-MS/MS method for the determination of 1-methyl-4-nitrosopiperazine (MNP) in multicomponent products with rifampicin - challenges and degradation studies

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Table S1. MNP content in the 3-component product - methods 1 and 2 comparison.

Method	MNP content [ppm]
Method 1 - Extraction with up to 5 mL of methanol	2.69
Method 2- extraction - water with the addition of NaOH /methanol	2.70

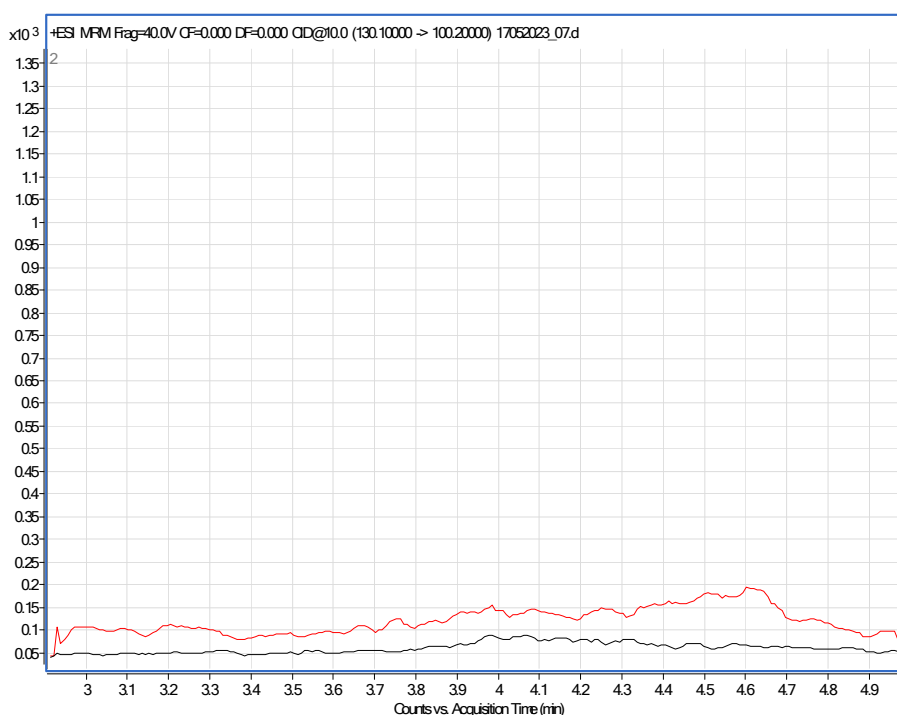


Figure S1. MRM chromatogram of blank for MNP (130.1 > 58.2 red curve, 130.1 > 100.2 black curve).

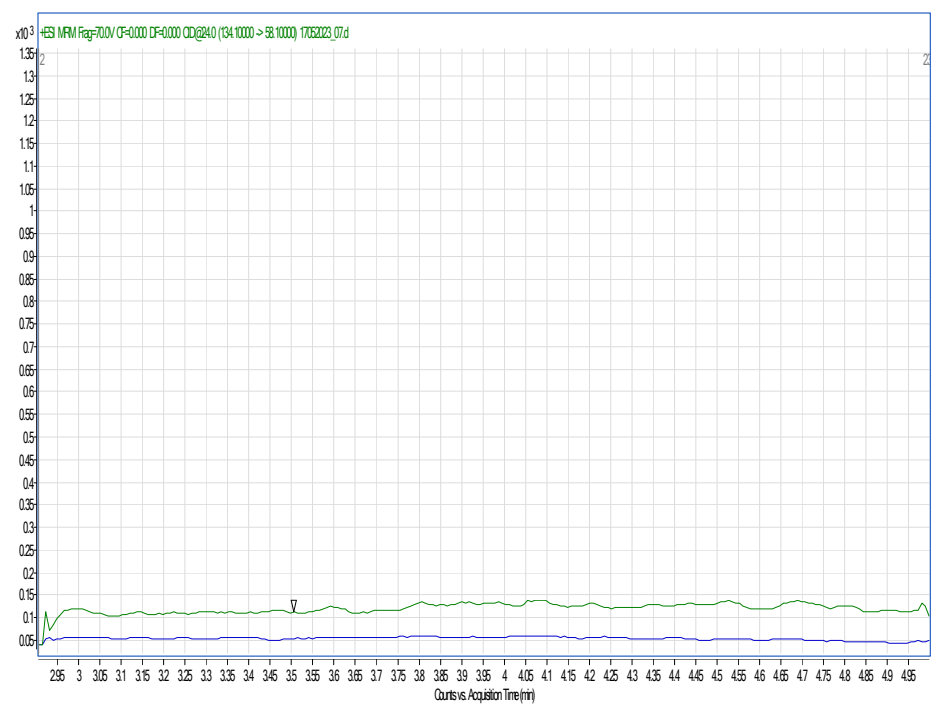


Figure S2. MRM chromatogram of blank for MNP-d4 (134.1 > 58.1 green curve, 134.1 > 104.1 blue curve).

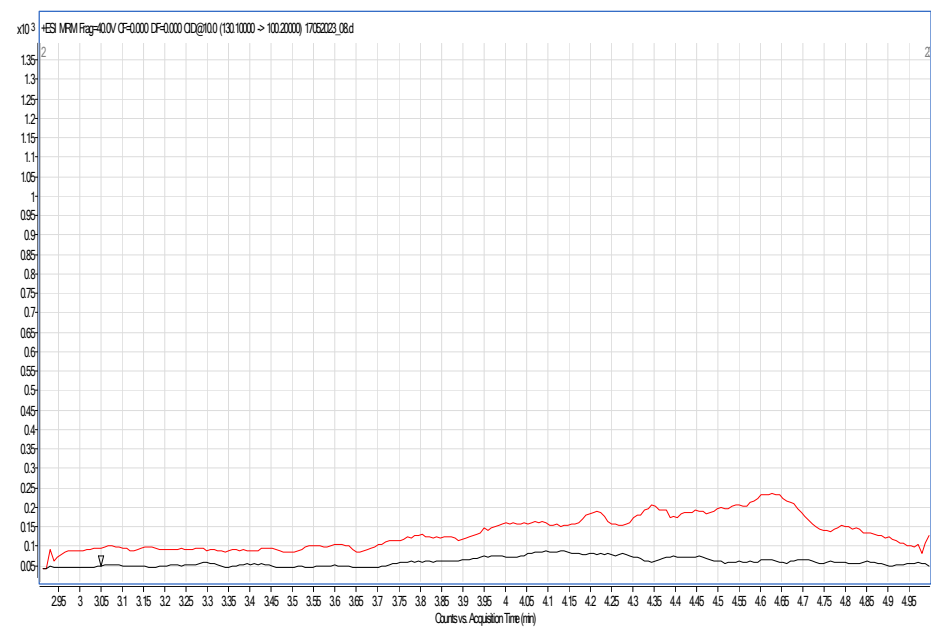


Figure S3. MRM chromatogram of blank solution spiked with the internal standard solution MNP-d4 for MNP (130.1 > 58.2 red curve, 130.1 > 100.2 black curve).

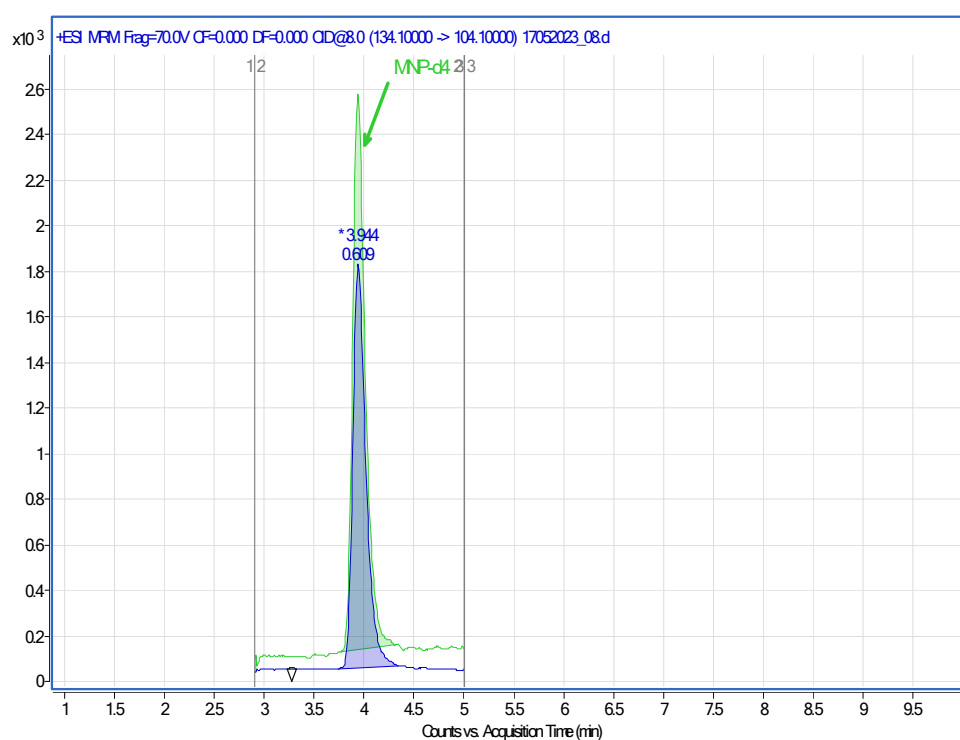


Figure S4. MRM chromatogram of blank solution spiked with the internal standard solution MNP-d4: MNP-d4 (134.1 > 58.1 green curve, 134.1 > 104.1 blue curve).

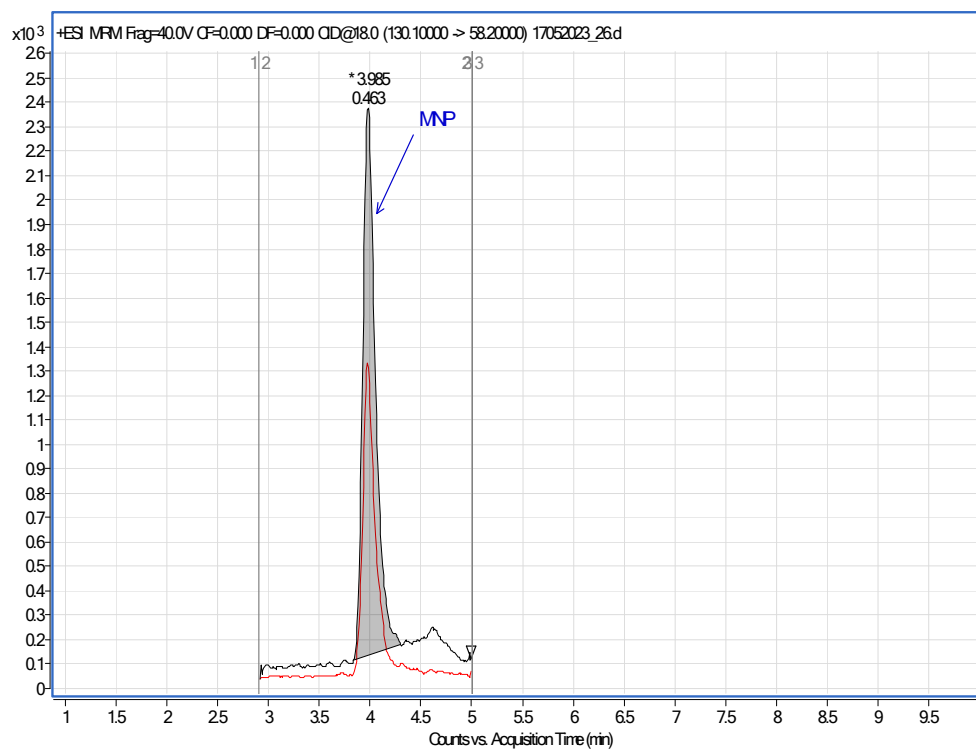


Figure S5. MRM chromatogram of standard solution of at 100% concentration level for MNP (130.1 > 100.2 red curve, 130.1 > 58.2 black curve).

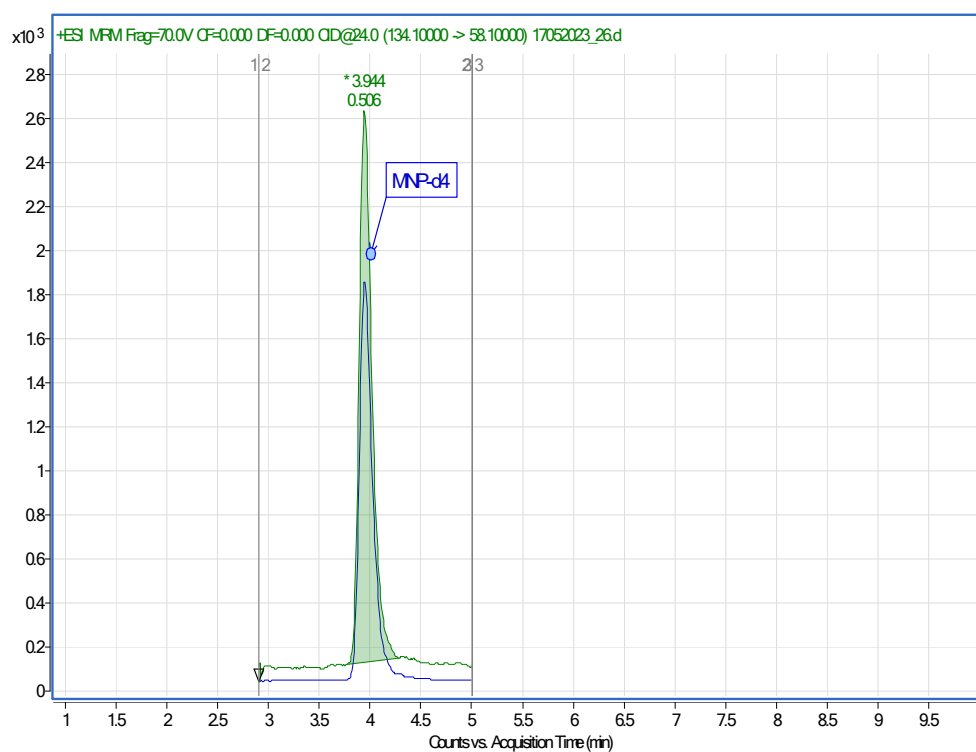


Figure S6. MRM chromatogram of standard solution of at 100% concentration level for MNP-d4 (134.1 > 58.1 green curve, 134.1 > 104.1 blue curve).

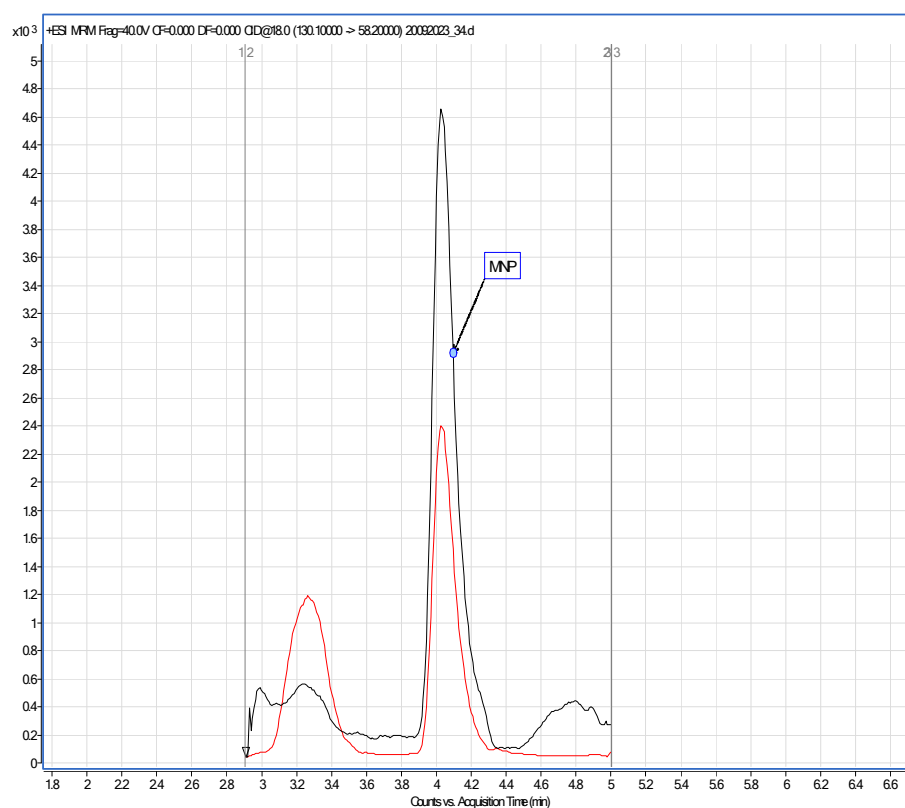


Figure S7. MRM chromatogram of sample solution of drug product (Rifampicin product four-component) for MNP (130.1 > 100.2 red curve, 130.1 > 58.2 black curve).

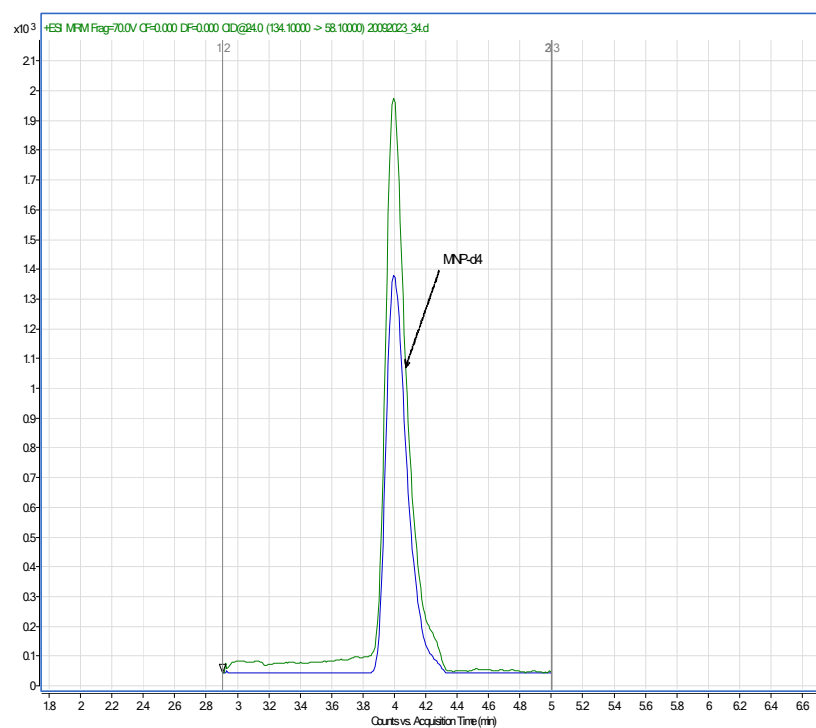


Figure S8. MRM chromatogram of sample solution of drug product (Rifampicin product four-component) for MNP-d4 (134.1 > 58.1 green curve, 134.1 > 104.1 blue curve).

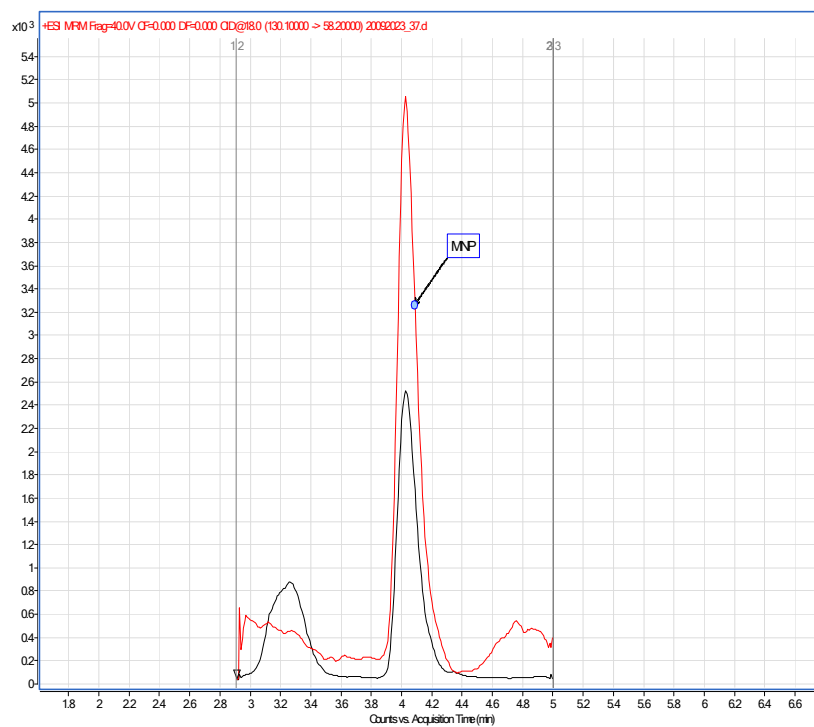


Figure S9. MRM chromatogram of reference solution (sample solution spiked with standard solution of MNP at concentration level of 17.22 ng/mL (5 ppm in respect to sample preparation) for MNP (130.1 > 58.2 red curve, 130.1 > 100.2 black curve).

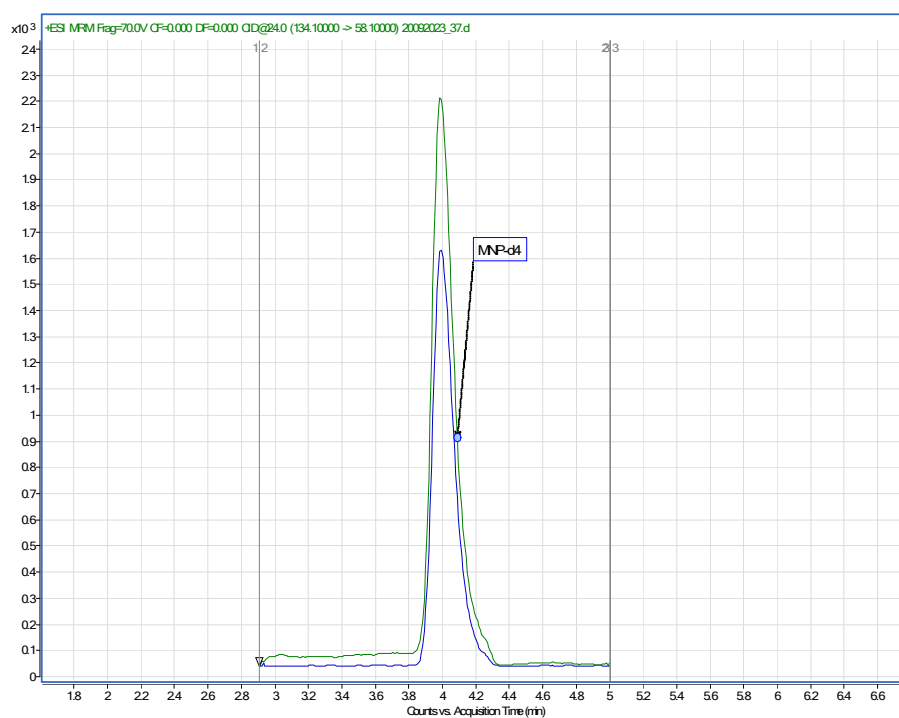


Figure S10. MRM chromatogram of reference solution (sample solution spiked with a standard solution of MNP at the concentration level of 17.22 ng/mL (5 ppm in respect to sample preparation) for MNP-d4 (134.1 > 58.1 green curve, 134.1 > 58.1 blue curve).

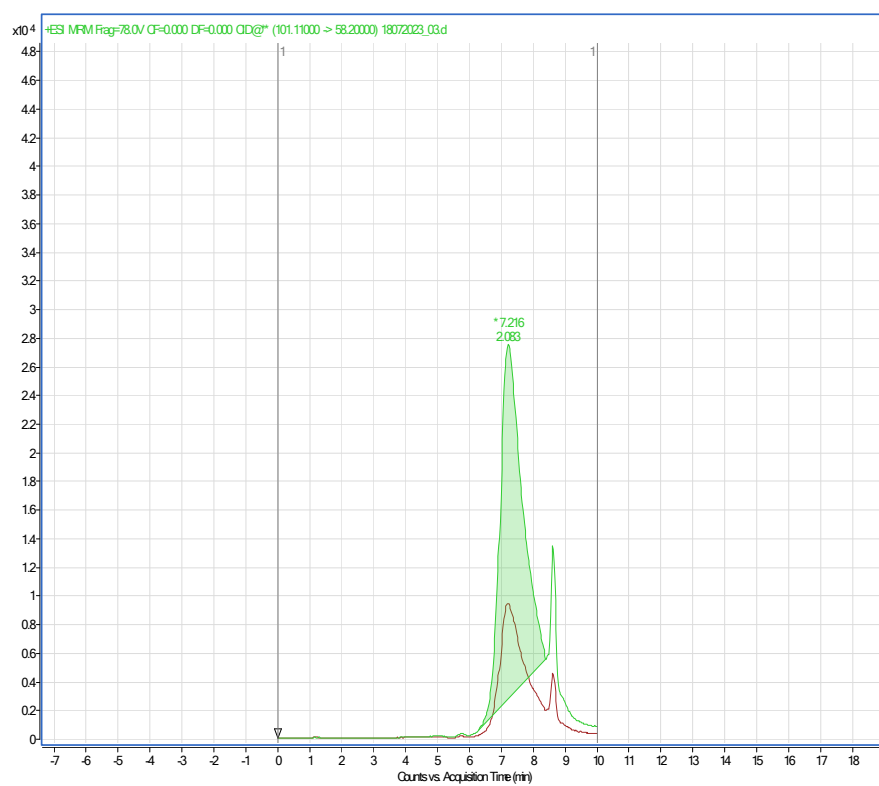


Figure S11. MRM chromatogram of 1-methyl-piperazine solution (101.1 > 58.2 green curve, 101.1 > 44.2 brown curve).

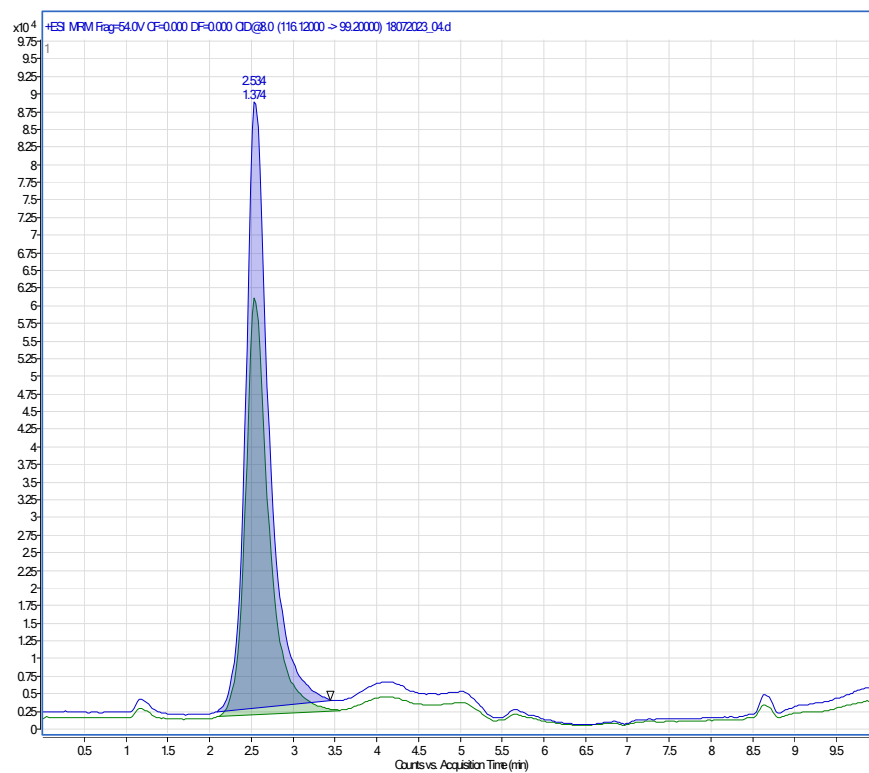


Figure S12. MRM chromatogram of 1-amino-4methyl-piperazine solution (116.12 > 99.2 blue curve, 116.12 > 56.1 green curve).

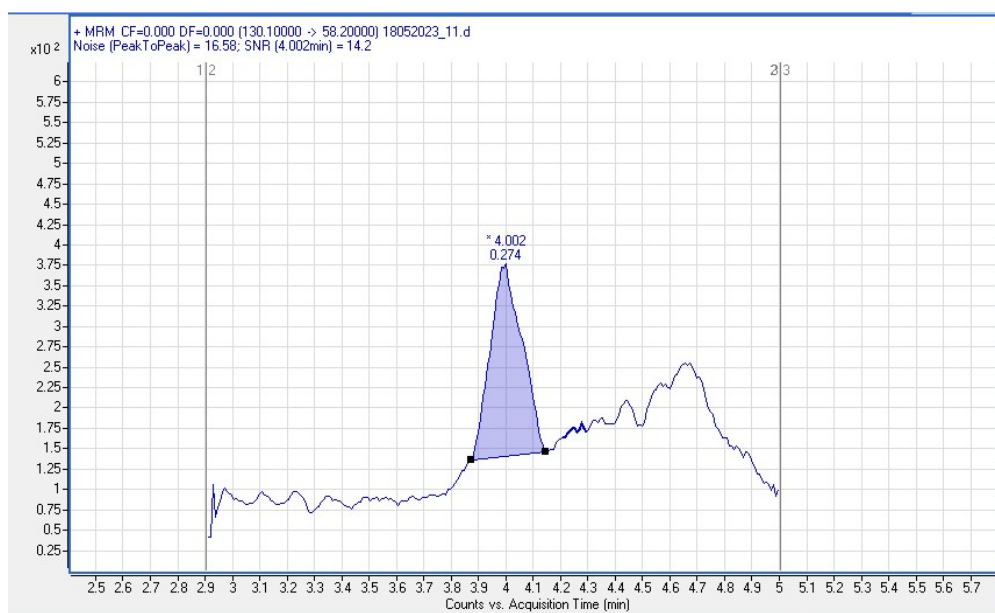


Figure S13. Chromatogram of LOQ solution of MNP 1.52 ppb.

Table S3. Results of examined reference solution with MNP at 100% concentration level.

Time	Peak area MNP	Peak area MNP-d4	Area MNP/Area MNP-d4 (Q)
0 h	19737	22144	0.891
0 h	19566	22213	0.881
8h	20916	23626	0.885
8h	20965	23426	0.895
24 h	20936	23666	0.885
24 h	20096	23094	0.870
		Mean	0.885
		SD	0.009
		RSD%	0.978

Table S4. Results of examined LOQ solution.

Time	Peak area MNP	Peak area MNP-d4	Area MNP/Area MNP-d4 (Q)
0 h	1988	0.090	0.090
0 h	1936	0.086	0.086
24 h	1935	0.082	0.082
24 h	1901	0.080	0.080
		Mean	0.085
		SD	0.004
		RSD%	5.048

Table S5. Results of sample solution.

Time	Peak area MNP	Peak area MNP-d4	Area MNP/Area MNP-d4(Q)
0 h	8274	10287	0.804
0 h	7241	8938	0.810
4 h	7796	9735	0.801
8 h	7426	8441	0.880
		Mean	0.824
		SD	0.038
		RSD%	4.556

Table S6. MS summary results for MNP and 1-amino-4-methylpiperazine.

Compound name	RT, min	Precursor ion, m/z [M+H] ⁺	Theoretical values, m/z	Predicted formula	Err, ppm	mSigma	Product ion (rel.int., %)
1-amino-4-methylpiperazine	3.1	116.1185	116.1182	C ₅ H ₁₃ N ₃	-2.2	15.7	99.0916 (100.00%) 84.0694 (7.25%) 70.0694 (5.21%) 56.0502 (11.24%)
N-methyl_N'-nitrosopiperazine	4.4	130.0977	130.0975	C ₅ H ₁₂ N ₃ O	-4.6	17.5	128.0800 (65.02%) 100.0997 (100.00%) 58.0651 (11.22%)

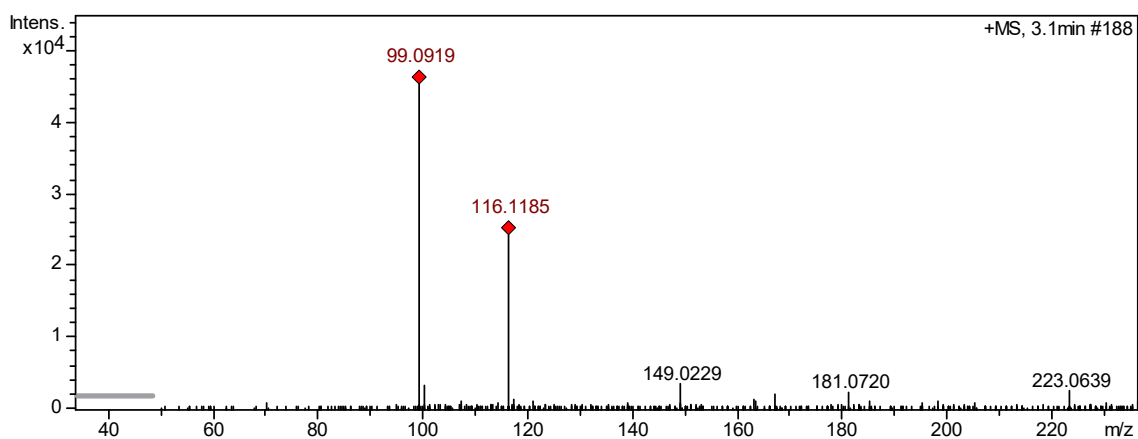


Figure S15. MS spectrum of 1-amino-4-methylpiperazine.

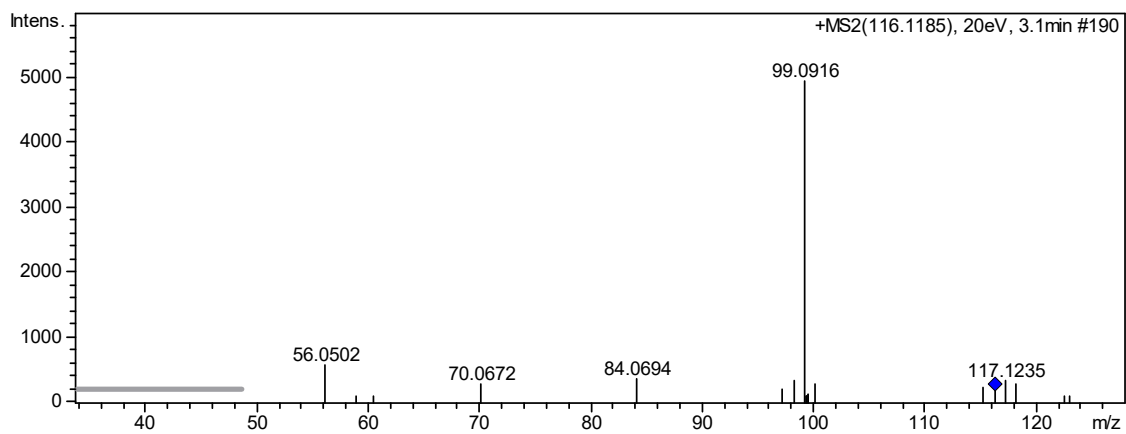


Figure S16. MS/MS spectrum of 1-amino-4-methylpiperazine.

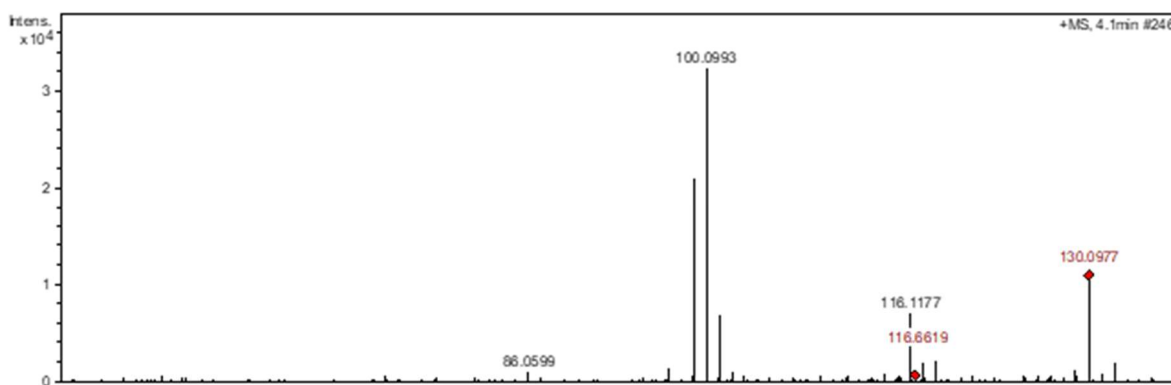


Figure S17. MS spectrum of MNP.

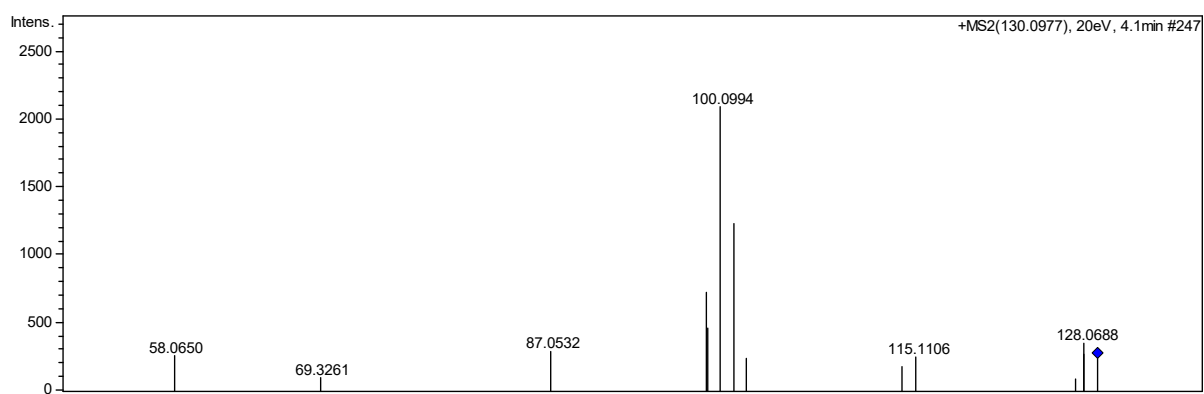


Figure S18. MS/MS spectrum of MNP.