25. BÖLÜM

Yeni Psikoaktif Maddeler ve Metabolitlerinin Tanımlanması

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Giriş

Yeni psikoaktif madde (new psychoactive substances, NPS), saf halde ya da müstahzar halinde, 1961 Tek Narkotik Sözleşmesi ve 1971 Psikotrop Maddeler Sözleşmesi ile kontrol edilmeyen, ancak halk sağlığı açısından benzer tehditler oluşturabilecek ve istismar edilen maddeler olarak tanımlanmaktadır. Buradaki "yeni" terimi maddenin mutlaka yeni sentezlendiği anlamına gelmez, yıllar önce ilk kez sentezlendiği halde uyuşturucu madde piyasasında yeni görülmeye başlanan maddeler olduğu da bilinmektedir(1). Bu maddelerin çoğu, esrar, eroin, kokain, amfetamin gibi yasadışı maddelerin yasal ikameleri olarak pazarlanmaktadır(2).

NPS piyasası sürekli eklenen yeni maddelerle hızla büyümeye ve değişmeye devam etmektedir. 2020 Avrupa Uyuşturucu Raporuna göre AB Erken Uyarı Sistemi tarafından yılda 50'den fazla NPS ilk kez tespit edilmiştir. Ayrıca daha önceden rapor edilen yaklaşık 400 NPS Avrupa pazarında görülmeye devam etmiştir. Bu maddeler, katinonlar, kannabinoidler, fenetilaminler, opioidler, triptaminler, benzodiazepinler gibi birçok farklı gruptan olsalar da, NPS piyasasında baskın olan grup katinonlar ve kannabinoidlerdir(3). NPS piyasasındaki bu dinamik durumun ana sebeplerinden birisi, kontrol altındaki maddelerin listelerle belirlenmesi durumunda, liste dışı olanların yasal olması

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nırken en iyi tercihler genellikle Faz I metabolitleridir. Analitik cihazda oluşturdukları dedektör cevabı daha yüksektir ve örnek içerisinde Faz II metabolitlerine göre daha stabildir. İdrar örnekleri analiz öncesi enzim ile ya da enzimsiz olarak hidroliz edilerek Faz I metabolitlerinin analizdeki alan değerleri arttırılabilir.

Sonuç

NPS'lerin tespiti ve güvenilir tanımlanması, toksikolojik analizler için metabolitlerinin belirlenmesi, biyolojik sıvılarda NPS ve metabolitlerinin tespiti için analitik yöntem geliştirme, kimyasal yapılarındaki benzerlikler, ortak analitik kütüphanelerde spektrumlarının olmaması, referans materyallerin sınırlı olması gibi nedenlerle, adli bilimciler için zorlayıcı olmaktadır. Ancak yeni çıkan NPSlerin tanımlanması ve sonrasında metabolizma çalışmalarının yapılması için kapsamlı, güvenilir, güçlü metotlar gerekmektedir. Bu bölümde NPSlerin öncelikle biyolojik olmayan matrislerde tanımlanması ve sonra toksikolojik analiz için metabolitlerinin belirlenmesi için analitik stratejiler sunuldu. Materyal içindeki NPSlerin tanımlanmasında düşük veya yüksek çözünürlüklü kütle spektrometrik yöntemlerle birlikte NMR ve/veya FTIR spektrumları ile daha detaylı yapı aydınlatması sağlanırken, metabolizma karakterizasyonu için *in vitro* ve *in vivo* yöntemler karşılaştırılarak tahmini metabolitlerin yapı tayini için LC-HR/MS tekniklerinin sağladığı kolaylıklardan bahsedildi.

KAYNAKLAR

1. UNODC, *What are NPS?*16/06/2021tarihinde www.unodc.org/LSS/Page/NPS adresinden ulaşılmıştır)
2. European Drug Report(2021).EMCDDA, (18/06/2021 tarihinde www.emcdda.europa.eu/system/files/publications/8585/20181816_TDAT18001ENN_PDF.pdf adresinden ulaşılmıştır)
3. World Drug Report(2020), UNODC. (18/06/2021 tarihindewww.unodc.org/unodc/en/data-and-analysis/wdr2021.html adresinden ulaşılmıştır)
4. Current_NPS_Threats_Vol.3.UNODC (18/06/2021tarihindewww.unodc.org/documents/scientific/Current_NPS_Threats_Vol.3.pdf adresinden ulaşılmıştır)
5. Archer RP, Treble R, Williams K.,Reference materials for new psychoactive substances. Drug Test and Anal. 2011;3(7-8):505-14.
6. SWGDRUGRecommendations(18/06/2021 tarihinde www.swgdrug.org/Documents/SWGDRUG%20Recommendations%20Version%208_FINAL_ForPosting_092919.pdf adresinden ulaşılmıştır)
7. UNODC , RecommendedmethodsfortheIdentificationandAnalysisofSyntheticCathinonesin Seized Materials(18/06/2021 tarihinde www.unodc.org/documents/scientific/Recommended_methods_for_the_Identification_and_Analysis_of_Synthetic_Cathinones_in_Seized_Materials-Rev..pdf adresinden ulaşılmıştır)
8. UNODC, Recommended Methods for the Identification and Analysis of Synthetic Cannabinoid Receptor Agonists in Seized Materials. (18/06/2021tarihindewww.unodc.org/documents/scientific/ STNAR48 _Rev.1_ebook.pdf adresinden ulaşılmıştır)

9. Reitzel LA, Dalsgaard PW, Müller IB, et al. Identification of ten new designer drugs by GC-MS, UPLC-QTOF-MS, and NMR as part of a police investigation of a Danish internet company. Drug Test Anal. 2012;4(5):342-54.
10. Uchiyama N, Kikura-Hanajiri R, Kawahara N, et al. Identification of a cannabinoid analog as a new type of designer drug in a herbal product. Chem Pharm Bull 2009;57(4):439-41.
11. Moosmann B, Kneisel S, Girreser U, et al. Separation and structural characterization of the synthetic cannabi-noidsJWH-412 and 1-[(5-fluoropentyl)-1H-indol-3yl]-(4-methyl-naphthalen-1-yl)methanone using GC-MS, NMR analysis and a flash chromatography system. Forensic Sci Int. 2012;220(1-3):e17-22.
12. Park, Y., Lee, C., Lee, H. et al. Identification of a new synthetic cannabinoid in a herbal mixture: 1-butyl-3-(2-methoxybenzoyl)indole. Forensic Toxicol (2013); 31, 187–196. https://doi.org/10.1007/s11419-012-0173-2
13. Shevyrin V, Melkozerov V, Nevero A, et al. Analytical characterization of some synthetic cannabinoids, derivatives of indole-3-carboxylic acid. Forensic Sci Int. 2013;232(1-3):1-10.
14. Kennedy J, Shanks KG, Van Natta K, et al. Rapid screening and identification of novel psychoactive substances using PaperSpray interfaced to high resolution mass spectrometry. Clinical Mass Spectrometry. 2016;1:3-10.
15. Choi H, Heo S, Kim E,et al. Identification of (1-pentylindol-3-yl)-(2,2,3,3-tetramethylcyclopropyl)methanone and its 5-pentyl fluorinated analog in herbal incense seized for drug trafficking. Forensic Toxicol. 2013;31,86-92
16. Shevyrin V, Melkozerov V, Nevero A, et al. 3-Naphthoylindazoles and 2-naphthoylbenzoimidazoles as novel chemical groups of synthetic cannabinoids: Chemical structure elucidation, analytical characteristics and identification of the first representatives in smoke mixtures. Forensic Sci Int. 2014;242:72-80.
17. Lobo Vicente J, Chassaigne H, Holland MV, et al. Systematic analytical characterization of new psychoactive substances: A case study. Forensic Sci Int. 2016;265:107-15.
18. Yeter O. Identification of the Synthetic Cannabinoid 1-(4-cyanobutyl)-N-(2-phenylpropan-2-yl)-1H-indazole-3-carboxamide (CUMYL-4CN-BINACA) in Plant Material and Quantification in Post-Mortem Blood Samples. J Anal Toxicol. 2017;41(9):720-8.
19. Sekuła K, Zuba D. Structural elucidation and identification of a new derivative of phenethylamine using quadrupole time-of-flight mass spectrometry.Rapid Commun Mass Spectrom. 2013;30;27(18):2081-90.
20. Zuba D, Sekuła K, Buczek A. Identification and characterization of 2,5-dimethoxy-4-nitro-β-phenethylamine (2C-N)--a new member of 2C-series of designer drug. Forensic Sci Int. 2012;222(1-3):298-305.
21. Bulska E, Bachliński R, Cyrański MK,et al. Comprehensive Protocol for the Identification and Characterization of New Psychoactive Substances in the Service of Law Enforcement Agencies. FrontChem. 2020; 8,693.
22. Ojanperä I, Kolmonen M, Pelander A. Current use of high-resolution mass spectrometry in drug screening relevant to clinical and forensic toxicology and doping control. Anal Bioanal Chem. 2012;403(5):1203-20.
23. Ramanathan R, Jemal M, Ramagiri S,et al. It is time for a paradigm shift in drug discovery bioanalysis: from SRM to HRMS. J Mass Spectrom.2011;46(6):595-601.
24. Franciso Javier Arrebola-Liébanas, Roberto Romero-González, Antonia Garrido Frenich, (2017) Chapter 1 - HRMS: Fundamentals and Basic Concepts,Editor(s): Roberto Romero-González, Antonia Garrido Frenich,Applications in High Resolution Mass Spectrometry, Elsevier,
25. Grabenauer M, Krol WL, Wiley JL, et al. Analysis of synthetic cannabinoids using high-resolution mass spectrometry and mass defect filtering: implications for nontargeted screening of designer drugs. Anal Chem. 012;84(13):5574-81.

26. Emwas A-H. The Strengths and Weaknesses of NMR Spectroscopy and Mass Spectrometry with Particular Focus on Metabolomics Research. İçinde: Methods in molecular biology (Clifton, NJ). 2015. s. 161-93.
27. Wille SMR, Raes E, Lillsunde P, et al. Relationship between oral fluid and blood concentrations of drugs of abuse in drivers suspected of driving under the influence of drugs.Ther Drug Monit.2009;31(4):511-9.
28. Langel K, Gjerde H, Favretto D, et al. Comparison of drug concentrations between whole blood and oral fluid. Drug Test Anal. 2014;6(5):461-71.
29. Pichini S, Rotolo MC, García J, et al. Neonatal withdrawal syndrome after chronic maternal consumption of 4-methylethcathinone. Forensic Sci Int. 2014;245:e33-35.
30. Nemeškalová A, Bursová M, Sýkora D, et al. Salting out assisted liquid-liquid extraction for liquid chromatography tandem-mass spectrometry determination of amphetamine-like stimulants in meconium. J Pharm Biomed Anal. 2019;172:42-9.
31. López-Rabuñal Á, Lendoiro E, Concheiro M, et al.A LC-MS/MS method for the determination of common synthetic cathinones in meconium. J Chromatogr B Analyt Technol Biomed Life Sci. 2019;1124:349-55.
32. Woźniak MK, Banaszkiewicz L, Wiergowski M, et al. Development and validation of a GC–MS/MS method for the determination of 11 amphetamines and 34 synthetic cathinones in whole blood. Forensic Toxicol. 2020;38(1):42-58.
33. Ivanov ID, Stoykova S, Ivanova E, et al. A case of 5F-ADB / FUB-AMB abuse: Drug-induced or drug-related death? Forensic Sci Int. 2019;297:372-7.
34. Mesihää S, Rasanen I, Pelander A, et al. Quantitative Estimation of 38 Illicit Psychostimulants in Blood by GC-APCI-QTOFMS with Nitrogen Chemiluminescence Detection Based on Three External Calibrators. J Anal Toxicol. 2020;44(2):163-72.
35. Bidny S, Gago K, Chung P, et al. Simultaneous Screening and Quantification of Basic, Neutral and Acidic Drugs in Blood Using UPLC-QTOF-MS. J Anal Toxicol. 2017 1;41(3):181-195.
36. Adamowicz P, Tokarczyk B. Simple and rapid screening procedure for 143 new psychoactive substances by liquid chromatography-tandem mass spectrometry. Drug Test Anal. 2016;8(7):652-67.
37. Toennes SW, Kauert GF. Excretion and detection of cathinone, cathine, and phenylpropanolamine in urine after kath chewing. Clin Chem. 2002;48(10):1715-9.
38. Scheidweiler, K.B., Jarvis, M.J.Y. & Huestis, M.A. Nontargeted SWATH acquisition for identifying 47 synthetic cannabinoid metabolites in human urine by liquid chromatography-high-resolution tandem mass spectrometry. Anal Bioanal Chem 407, 883–897 (2015).
39. Ishii A, Kusakabe K, Kato N, et al. Studies on the phase I metabolites of the new designer drug 1-(2,3-dihydro-1H-inden-5-yl)-2-(pyrrolidine-1-yl)butan-1-one (5-PPDI) in human urine. Forensic Sci Int. 2020;310:110214.
40. Staeheli SN, Veloso VP, Bovens M, et al. Liquid chromatography-tandem mass spectrometry screening method using information-dependent acquisition of enhanced product ion mass spectra for synthetic cannabinoids including metabolites in urine. Drug Test Anal. 2019;11(9):1369-1376.
41. Lehmann, S., Schulze, B., Thomas, A. et al. Organ distribution of 4-MEC, MDPV, methoxetamine and α-PVP: comparison of QuEChERS and SPE. Forensic Toxicol 2018;36, 320–33.
42. Rouxinol D, Dias da Silva D, Silva JP, et al. Biodistribution and metabolic profile of 3,4-dimethylmethcathinone (3,4-DMMC) in Wistar rats through gas chromatography-mass spectrometry (GC-MS) analysis. Toxicol Lett. 2020;320:113-23.
43. Margalho C, Castanheira A, Real FC, et al. Determination of "new psychoactive substances" in postmortem matrices using microwave derivatization and gas chromatography-mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2016;1020:14-23.

44. Presley BC, Gurney SMR, Scott KS, et al. Metabolism and toxicological analysis of synthetic cannabinoids in biological fluids and tissues. Forensic Sci Rev. 2016;28(2):103-69.
45. Yeter O, Erol Öztürk Y. Detection and quantification of 5F-ADB and its methyl ester hydrolysis metabolite in fatal intoxication cases by liquid chromatography-high resolution mass spectrometry. Forensic Sci Int. 2019;302:109866.
46. Minakata K, Yamagishi I, Nozawa H, et al. Sensitive identification and quantitation of parent forms of six synthetic cannabinoids in urine samples of human cadavers by liquid chromatography–tandem mass spectrometry. Forensic Toxicol. 2017;35(2):275-83.
47. Yeter O. Simultaneous determination of 4F-MDMB BINACA, a new synthetic cannabinoid, and its metabolites in human blood samples by LC-MS/MS. Journal of the Turkish Chemical Society Section A: Chemistry. 2020;7(3):827-32.
48. Wagmann L, Maurer HH. Bioanalytical Methods for New Psychoactive Substances. Handb Exp Pharmacol. 2018;252:413-39.
49. Zhu Y, Li L, Zhang G, et al. Metabolic characterization of pyrotinib in humans by ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2016;1033-1034:117-27.
50. Diao X, Huestis MA. New Synthetic Cannabinoids Metabolism and Strategies to Best Identify Optimal Marker Metabolites. Front Chem. 2019;https://doi.org/10.3389/fchem.2019.00109
51. Costa A, Sarmento B, Seabra V. An evaluation of the latest in vitro tools for drug metabolism studies. Expert Opin Drug Metab Toxicol. 2014;10(1):103-19.
52. Meyer MR. New psychoactive substances: an overview on recent publications on their toxicodynamics and toxicokinetics. Arch Toxicol. 2016;90(10):2421-44.
53. Wohlfarth A, Gandhi AS, Pang S, et al. Metabolism of synthetic cannabinoids PB-22 and its 5-fluoro analog, 5F-PB-22, by human hepatocyte incubation and high-resolution mass spectrometry. Anal Bioanal Chem. 2014;406(6):1763-80.
54. Castaneto MS, Wohlfarth A, Pang S, et al. Identification of AB-FUBINACA metabolites in human hepatocytes and urine using high-resolution mass spectrometry. Forensic Toxicol. 2015;2(33):295-310.
55. Diao X, Carlier J, Zhu M, et al. Human Hepatocyte Metabolism of Novel Synthetic Cannabinoids MN-18 and Its 5-Fluoro Analog 5F-MN-18. Clinical Chemistry. 2017;63(11):1753-63.
56. Richter LHJ, Maurer HH, Meyer MR. New psychoactive substances: Studies on the metabolism of XLR-11, AB-PINACA, FUB-PB-22, 4-methoxy-α-PVP, 25-I-NBOMe, and meclonazepam using human liver preparations in comparison to primary human hepatocytes, and human urine. Toxicol Lett. 2017;280:142-50.
57. Castell JV, Jover R, Martínez-Jiménez CP, et al. Hepatocyte cell lines: their use, scope and limitations in drug metabolism studies. Expert Opin Drug Metab Toxicol. 2006;2(2):183-212.
58. Mogler L, Wilde M, Huppertz LM, et al. Auwärter V. Phase I metabolism of the recently emerged synthetic cannabinoid CUMYL-PEGACLONE and detection in human urine samples. Drug Test Anal. 2018;10(5):886-91.
59. Öztürk YE, Yeter O, Öztürk S, et al. Detection of metabolites of the new synthetic cannabinoid CUMYL-4CN-BINACA in authentic urine samples and human liver microsomes using high-resolution mass spectrometry. Drug Test Anal.2018;10(3):449-59.
60. Erol Ozturk Y, Yeter O. In Vitro Phase I Metabolism of the Recently Emerged Synthetic MDMB-4en-PINACA and Its Detection in Human Urine Samples. J Anal Toxicol. 2021;44(9):976-84.
61. Yeter O, Ozturk YE. Metabolic profiling of synthetic cannabinoid 5F-ADB by human liver microsome incubations and urine samples using high-resolution mass spectrometry. Drug Test Anal. 2019;11(6):847-58.

62. Åstrand A, Vikingsson S, Lindstedt D, et al. Metabolism study for CUMYL-4CN-BINACA in human hepatocytes and authentic urine specimens: Free cyanide is formed during the main metabolic pathway. Drug Test Anal. 2018; doi: 10.1002/dta.2373
63. Meyer MR.(2017), Toxicokinetics of NPS: Update 2017. Maurer HH, Brandt SD, editörler. New Psychoactive Substances : Pharmacology, Clinical, Forensic and Analytical Toxicology, Springer International Publishing;441-59. (Handbook of Experimental Pharmacology).
64. de Souza Anselmo C, Sardela VF, Matias BF, de Carvalho AR, de Sousa VP, Pereira HMG, vd. Is zebrafish (Danio rerio) a tool for human-like metabolism study? Drug Test Anal. Kasım 2017;9(11-12):1685-94.
65. Richter LHJ, Herrmann J, Andreas A, et al. Tools for studying the metabolism of new psychoactive substances for toxicological screening purposes - A comparative study using pooled human liver S9, HepaRG cells, and zebrafish larvae. Toxicol Lett. 2019;305:73-80.
66. Richeval C, Gicquel T, Hugbart C,et al. In vitro Characterization of NPS Metabolites Produced by Human Liver Microsomes and the HepaRG Cell Line Using Liquid Chromatographyhigh Resolution Mass Spectrometry (LC-HRMS) Analysis: Application to Furanyl Fentanyl. CPB. 2018;18(10):806-14.
67. Asha S, Vidyavathi M. Role of human liver microsomes in in vitro metabolism of drugs-a review. Appl Biochem Biotechnol. 2010;160(6):1699-722.
68. Bickett DJ, MacKenzie PI, Veronese ME, et al. In vitro approaches can predict human drug metabolism. Trends Pharmacol Sci. 1993;14(8):292-4.
69. Williams JA, Hyland R, Jones BC, et al. Drug-drug interactions for UDP-glucuronosyltransferase substrates: a pharmacokinetic explanation for typically observed low exposure (AUCi/AUC) ratios. Drug Metab Dispos. 2004;32(11):1201-8.
70. Sidelmann UG, Cornett C, Tjørnelund J, et al. A comparative study of precision cut liver slices, hepatocytes, and liver microsomes from the Wistar rat using metronidazole as a model substance. Xenobiotica. 1996;26(7):709-22.
71. Mae T, Inaba T, Konishi E, et al. Identification of enzymes responsible for rifalazil metabolism in human liver microsomes. Xenobiotica. 2000;30(6):565-74.
72. Mandagere AK, Thompson TN, Hwang KK. Graphical model for estimating oral bioavailability of drugs in humans and other species from their Caco-2 permeability and in vitro liver enzyme metabolic stability rates. J Med Chem. 2002 Jan 17;45(2):304-11
73. Sumida K, Ooe N, Nagahori H, et al. An in Vitro Reporter Gene Assay Method Incorporating Metabolic Activation with Human and Rat S9 or Liver Microsomes. Biochem Biophys Res Commun. 2001;280(1):85-91.
74. Hutter M, Moosmann B, Kneisel S, et al. Characteristics of the designer drug and synthetic cannabinoid receptor agonist AM-2201 regarding its chemistry and metabolism. J Mass Spectrom 2013;48(7):885-94.
75. Kevin RC, Lefever TW, Snyder RW, et al. Kinetic and metabolic profiles of synthetic cannabinoids NNEI and MN-18. Drug Test Anal. 2018;10(1):137-47.
76. Gandhi AS, Wohlfarth A, Zhu M, et al. High-resolution mass spectrometric metabolite profiling of a novel synthetic designer drug, N-(adamantan-1-yl)-1-(5-fluoropentyl)-1H-indole-3-carboxamide (STS-135), using cryopreserved human hepatocytes and assessment of metabolic stability with human liver microsomes. Drug Test Anal. 2015;7(3):187-98.
77. Diao X, Carlier J, Zhu M, et al. In vitro and in vivo human metabolism of a new synthetic cannabinoid NM-2201 (CBL-2201). Forensic Toxicol. 2017;35(1):20-32.
78. Carlier, J., Diao, X., Wohlfarth, A., et al. In Vitro Metabolite Profiling of ADB-FUBINACA, A New Synthetic Cannabinoid. Current neuropharmacology, 2017;15(5), 682–691.
79. Wohlfarth A, Pang S, Zhu M, et al. First metabolic profile of XLR-11, a novel synthetic cannabinoid, obtained by using human hepatocytes and high-resolution mass spectrometry. Clin Chem. 2013;59(11):1638-48.