

基于GC Orbitrap/MS技术的高效基因毒性杂质筛查分析

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关键词:

GC Orbitrap/MS; HRF; 基因毒性杂质

摘要

利用超高分辨气质联用GC Orbitrap/MS技术的高灵敏度特点, 结合基因毒性物质专属谱库建立了药物中微量基因毒性物质的非靶向筛查方法。方法高效, 高分辨过滤技术(HRF)能够快速锁定可疑物质; 同时方法准确, 通过EI及CI数据可锁定分子离子峰, 结合小于1ppm的质量精度, 可实现准确性。通过该方法对某原料药进行筛查, 最终在324个峰里快速筛查出N,N-二甲基苯胺、抗氧化剂264以及邻苯二甲酸二烯丙酯三种基因毒性杂质。

前言

基因毒性, 是指污染物能直接或间接损伤细胞DNA, 产生致突变和致癌作用的程度。西药往往因工艺、包材等原因可能在新药合成、原料纯化及储存运输过程中产生或迁移基因毒性物质, 例如甲磺酸奈非那非事件及亚硝胺事件。

药物中基因毒性物质的检测, 是药品质量的重要指标之一。因其危害之大, 含量及限量之低, 加之种类繁多, 需要跨技术平台进行高灵敏度的综合分析。对于气质联用技术而言, 中国药典及美国FDA出台了HS-GCMS及GCMSMS方法检测药物中亚硝胺的方法标准。新加坡政府更是推出二甲双胍中NDMA的GC Orbitrap/MS方法标准《Determination of N-nitrosodimethylamine(NDMA)in Metformin Products by HRAM-GCMS》。其中, 低分辨气质联用检测微量基因毒性物质为靶向分析, 需要特征离子/离子对信息, 开展大规模基毒的筛查需要不断扩充数据库, 且无法进行数据库之外的溯源分析。GC Orbitrap/MS低至fg级别的灵敏度, 结合全扫描的无偏向性分析无疑是同时定性与定量完美解决方案。除此之外, 赛默飞在美国国立卫生院(NIH)提出的共计1547种具有直接或潜在基因毒性的物质基础上, 开发了适合气相分析的927种基毒5841张谱图的谱库, 增强了检索的专一性和指向性, 能够更好地锁定化合物。而非通过NIST、Wiley等谱库的“海底捞针”式检索。

本文利用赛默飞超高分辨质谱GC Orbitrap/MS结合基因毒性物质的专有谱库, 对某原料药进行了基因毒性物质的筛查和鉴定, 为药物中基因毒性物质的检测提供了又一有力、可靠的方法。

1. 实验部分

1.1 仪器

质谱仪: Exactive GC

气相色谱仪: Trace 1310 GC

自动进样器: Triplus RSH

色谱柱: TG-1MS(30 m×0.32mm×0.25μm)

1.2 分析条件

GC 条件

进样口: SSL进样口

恒流模式: 1.5mL/min

进样体积: 1μL

升温程序: 40 °C (6min)_22 °C/min_200 °C (5min)_25 °C/min_310 °C (5min)

MS条件

传输线温度: 250 °C

离子源温度: 280 °C

采集方式: 分辨率R=60000(FWHM, m/z 200)

Full Scan全扫描: 50-500m/z

1.3 样品处理

精密称取某原料药0.5g, 加DCM (二氯甲烷) 5ml, 涡旋1min, 超声15min, 4000rpm离心5min, 取上清液适量, 过0.45μm滤膜后直接进样分析。

2.结果讨论

2.1筛查流程

依据1.2建立的方法，分别采用EI及PCI采集样品（TIC图见图1），EI源采集的原始数据经Deconvolution plugin软件进行基于质谱优先的解卷积，而后进行谱库搜索。HRF技术可将实际采集的碎片离子与检索出化合物的理论碎片离子进行比较。如果实测图谱里面可以被解释的离子越多，高分辨质谱图过滤检索值（HRF）值越高，即此化合物可能性越大（HRF计算公式如下）。PCI源采集的数据则用于辅助确认。

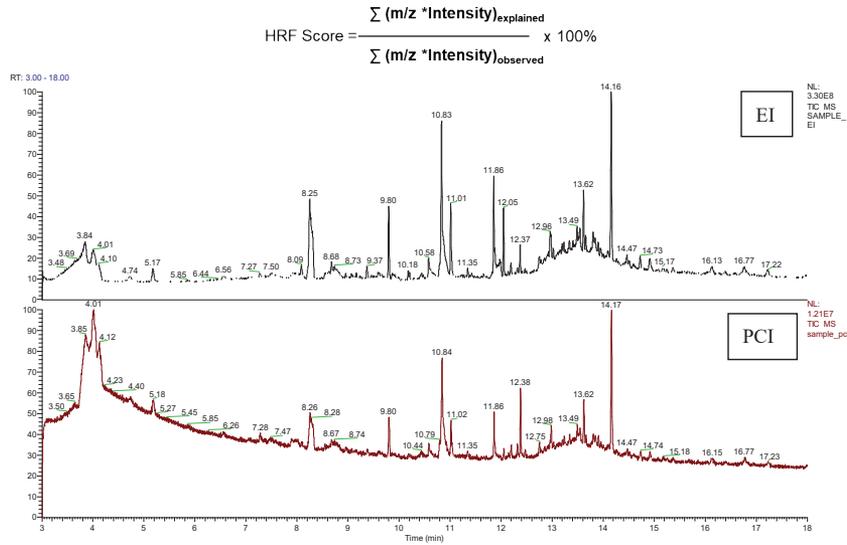


图1 EI及PCI下的全扫描色谱图

由图1可看出，样品基质相对复杂，经过解卷积、NIST谱库检索后获得324个峰，即便以SI>700、HRF>90对结果进行过滤，仍有72个峰，基毒筛查工作量巨大。而以基因毒性物质专属谱库进行检索，并以同样过滤条件筛选峰，仅有3个结果，如图2。



图2 检索结果对比

2.2结果讨论

Peak@RT8.732的定性

基于基毒谱库的首个检索结果为N,N-二甲基苯胺 (CAS#: 121-69-7)，而该化合物在以NIST谱库检索时，并非最高匹配度。通过对比其EI及PCI质谱图信息，可确认M⁺为121.08865，并且其与C₈H₁₁N理论分子离子峰质量偏差仅为0.36787ppm，M⁺与同位素M+2的比值也与软件计算的理论值相似。

Component Name	RT	Ref m/z	Score	Matched Compound	Formula	CAS	SI	HRF Score	RSI	RHRF Score	M+ m/z	Empirical M+	M+ Lib	% Elements
Benzenamine, N,N-dim...	8.732		96	Benzenamine, N,N-dim...	C8H11N	121-69-7	848	97.478	870	99.2903	121.088600	121.088631	Yes	100
Phenol 2,6-bis(1,1-dim...	11.878		95.6	Benzenamine, N,N-dim...	C8H11N	121-69-7	830	97.478	850	99.2903	121.088600	121.088631	Yes	100
1,2-Benzenedicarboxylic ...	12.976		95.5	BENZENAMINE, N,N-Di...	C8H11N	121-69-7	823	97.478	847	100	121.088600	121.088631	Yes	100
			95.4	Benzenamine, N,N-dim...	C8H11N	121-69-7	821	97.478	845	100	121.088600	121.088631	Yes	100
			95.4	Benzenamine, N,N-dim...	C8H11N	121-69-7	818	97.478	842	100	121.088600	121.088631	Yes	100
			95.4	Benzenamine, N,N-dim...	C8H11N	121-69-7	821	97.478	845	100	121.088600	121.088631	Yes	100
			95.3	Benzenamine, N,N-dim...	C8H11N	121-69-7	814	97.478	854	100	121.088600	121.088631	Yes	100
			95.2	Benzenamine, N,N-dim...	C8H11N	121-69-7	812	97.478	912	100	121.088600	121.088631	Yes	100
			95	Benzenamine, N,N-dim...	C8H11N	121-69-7	802	97.478	838	99.267	121.088600	121.088631	Yes	100
			94.9	Benzenamine, N,N-dim...	C8H11N	121-69-7	797	97.478	824	99.2822	121.088600	121.088631	Yes	100
			94.6	Benzenamine, N,N-dim...	C8H11N	121-69-7	782	97.478	832	100	121.088600	121.088631	Yes	100
			94	Benzenamine, 2,6-dim...	C8H11N	87-62-7	750	97.478	768	99.2903	121.088600	121.088631	Yes	100
			93.7	Benzenamine, 2,6-dim...	C8H11N	87-62-7	735	97.478	751	99.2903	121.088600	121.088631	Yes	100
			93.6	Diazenesulfonic acid, [4-(-...]	C8H11N2NaO3S	140-56-7	718	98.1747	737	100	252.041332	NF	No	100
			93.5	Benzenamine, 2,6-dim...	C8H11N	87-62-7	726	97.478	743	99.2903	121.088600	121.088631	Yes	100
			93.4	Benzenamine, 2,6-dim...	C8H11N	87-62-7	721	97.478	738	99.2903	121.088600	121.088631	Yes	100

图3 peak@RT8.732基毒谱库检索结果

Component Name	RT	Ref m/z	Score	Matched Compound	Formula	CAS	SI	HRF Score	RSI	RHRF Score	M+ m/z	Empirical M+	M+ Lib	% Elements
1,3,3-Trimethoxybutane	4.008		95.3	Benzenamine, N,3-dim...	C8H11N	696-44-6	816	97.478	840	100	121.088600	121.088631	Yes	100
Succinic acid, di(4-chlor...	4.073		94.9	Benzenamine, N,N-dime...	C8H11N	121-69-7	797	97.478	824	99.2822	121.088600	121.088631	Yes	100
Decane, 2,5,6-trimethyl-	7.356		94.8	Benzenamine, N,4-dim...	C8H11N	623-08-5	789	97.478	809	99.2903	121.088600	121.088631	Yes	100
1-Decene, 3,4-dimethyl-	7.457		94.4	5,6,7,8-Tetrahydronadoliz...	C8H11N	13618-88-7	769	97.478	791	100	121.088600	121.088631	Yes	100
Mesitylene	7.468		93.8	1-Propanone, 1-phenyl-2...	C15H15NO	42787-38-2	727	98.1747	747	100	225.114815	NF	Yes	100
Benzene, 1-ethyl-2-meth...	7.540		93.7	4-Nitro-N-(2,6-xylyl)ben...	C14H14N2O4S	109508-84-1	723	98.1747	752	100	306.066878	NF	Yes	100
Hexane, 3,3-dimethyl-	7.886		93.6	Benzenemethanamine, N...	C8H11N	103-67-3	729	97.478	751	100	121.088600	121.088631	Yes	100
Oxalic acid, isobutyl non...	7.980		93.6	Benzenamine, 2,4-dim...	C8H11N	95-68-1	731	97.478	747	99.2903	121.088600	121.088631	Yes	100
Bicyclo[2.2.2]octa-2,5-di...	8.539		93.5	Benzenemethanol, o-(1-...	C15H17NO	42787-62-2	712	98.1747	731	100	227.130465	NF	Yes	100
Benzenamine, N,N-dime...	8.681		93.4	2-Butanone, 3-amino-4-...	C10H13NO	40513-35-7	705	98.1747	725	100	163.099165	NF	Yes	100
Benzenamine, N,3-dim...	8.732		93.2	1,2-Ethanediamine, N,N...	C16H20N2	7030-60-6	712	97.478	739	100	240.162100	NF	Yes	100
Benzene, tert-butyl-	9.121		93.2	Pyridine, 2-ethyl-6-meth...	C8H11N	1122-69-6	712	97.478	731	99.2903	121.088600	121.088631	Yes	100
Benzene, tert-butyl-	9.155		93.2	Benzenamine, 3,4-dim...	C8H11N	95-64-7	709	97.478	725	99.2903	121.088600	121.088631	Yes	100
1-(2-Pyridyl)-2,2-dimeth...	9.610													

图4 peak@RT8.732 NIST谱库检索结果

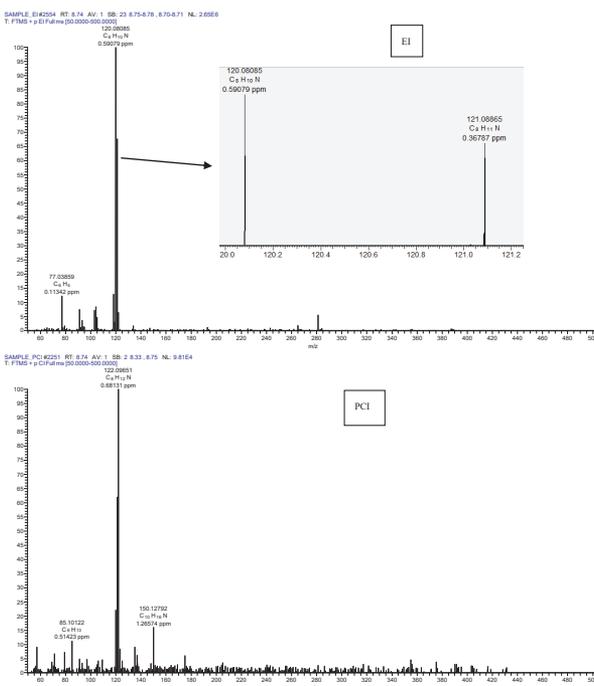


图5 EI及PCI质谱图对比

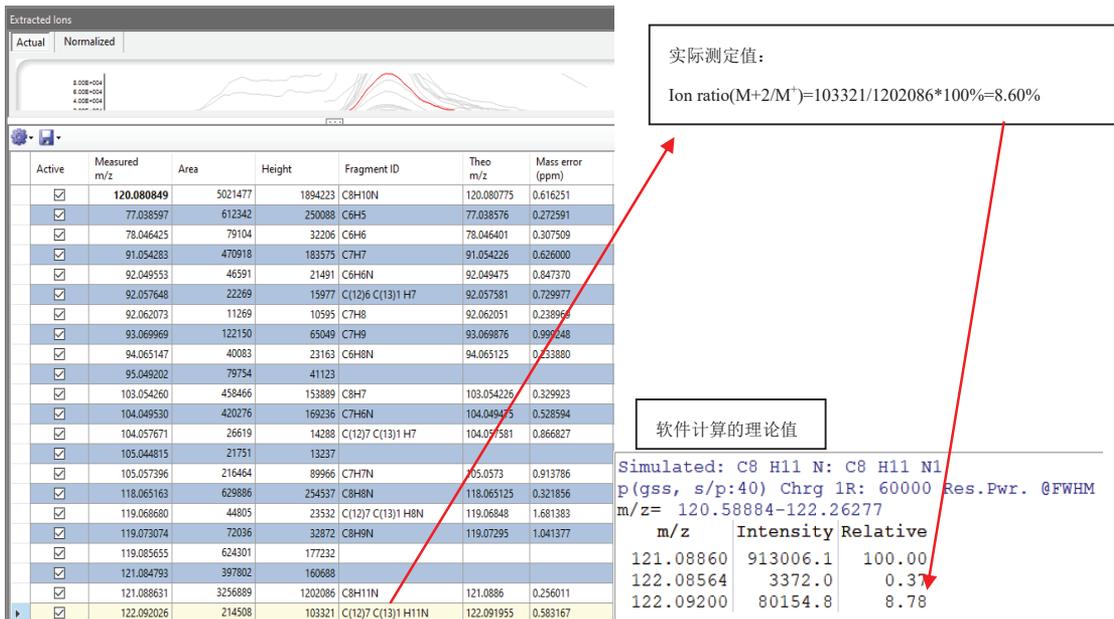


图6 同位素峰丰度对比

Peak@RT11.878的定性

基于基毒谱库的首个检索结果为抗氧化剂264 (CAS#: 128-37-0)，而该化合物在以NIST谱库检索时，排名第四。通过对比其EI及PCI质谱图信息，可确认M⁺为220.18235，并且其与C₁₅H₂₄O理论分子离子峰质量偏差仅为0.84629ppm，M⁺与同位素M+1比值也与软件计算的理论值相似。

Peak List: (3)

Component Name	RT	Ref m/z
Benzenamine, N,N-dime...	8.732	
Phenol, 2,6-bis(1,1-dimet...	11.878	
1,2-Benzenedicarboxylic ...	12.976	

Peak Identifications: (42)

Score	Matched Compound	Formula	CAS	SI	HRF Score	RSI	RHRF Score	M+ m/z	Empirical M+	M+ Lib	% Elements
98.2	Phenol, 2,6-bis(1,1-dim...	C15H24O	128-37-0	918	99.5013	965	100	220.182166	220.182327	Yes	100
98	Phenol, 2,6-bis(1,1-dimet...	C15H24O	128-37-0	907	99.5013	954	100	220.182166	220.182327	Yes	100
97.9	Phenol, 2,6-bis(1,1-dimet...	C15H24O	128-37-0	902	99.5013	948	100	220.182166	220.182327	Yes	100
97.7	Phenol, 2,6-bis(1,1-dimet...	C15H24O	128-37-0	896	99.5013	941	100	220.182166	220.182327	Yes	100
97.6	Phenol, 2,6-bis(1,1-dimet...	C15H24O	128-37-0	891	99.5013	896	99.7521	220.182166	220.182327	Yes	100
97.6	Phenol, 2,6-bis(1,1-dimet...	C15H24O	128-37-0	887	99.5013	914	100	220.182166	220.182327	Yes	100
97.5	Phenol, 2,6-bis(1,1-dimet...	C15H24O	128-37-0	886	99.5013	932	100	220.182166	220.182327	Yes	100
97.4	Phenol, 2,6-bis(1,1-dimet...	C15H24O	128-37-0	877	99.5013	902	100	220.182166	220.182327	Yes	100
97.4	Phenol, 2,6-bis(1,1-dimet...	C15H24O	128-37-0	880	99.5013	925	100	220.182166	220.182327	Yes	100
97.3	Phenol, 2,6-bis(1,1-dimet...	C15H24O	128-37-0	872	99.5013	893	99.7445	220.182166	220.182327	Yes	100
97.3	Phenol, 2,6-bis(1,1-dimet...	C15H24O	128-37-0	876	99.5013	920	100	220.182166	220.182327	Yes	100
97.2	Phenol, 2,6-bis(1,1-dimet...	C15H24O	128-37-0	870	99.5013	891	99.7445	220.182166	220.182327	Yes	100
96.9	Phenol, 2,6-bis(1,1-dimet...	C15H24O	128-37-0	853	99.5013	858	99.7521	220.182166	220.182327	Yes	100
96.8	Phenol, 2,6-bis(1,1-dimet...	C15H24O	128-37-0	850	99.5013	850	99.5013	220.182166	220.182327	Yes	100
96.8	Phenol, 2,6-bis(1,1-dimet...	C15H24O	128-37-0	851	99.5013	851	99.5013	220.182166	220.182327	Yes	100
96.7	Phenol, 2,6-bis(1,1-dimet...	C15H24O	128-37-0	845	99.5013	848	99.7442	220.182166	220.182327	Yes	100

图7 peak@RT11.878基毒的谱库检索结果

Peak List: (67)

Component Name	RT	Ref m/z
Phthalic acid, 4-isopropy...	11.386	
2-Phenyl-N-(2-pyridinyl...	11.400	
4'-Propoxy-2-methylpro...	11.519	
Benzene, 1-(1,1-dimethyl...	11.541	
2,4,6-Tris(1,1-dimethyl...	11.824	
2,4-Di-tert-butylphenol, ...	11.858	
2,4,6-Tris(1,1-dimethyl...	11.878	
Glutaric acid, but-3-yn-2...	12.186	
Diethyl Phthalate	12.195	
Dodecane, 2,7,10-trimeth...	12.312	
Cyclohexane, 1,1-dimeth...	12.410	
Undecane, 4,7-dimethyl...	12.464	
3-Butenyl adipate	12.745	
2,6-Diisooctylphthalate...	12.781	

Peak Identifications: (9)

Score	Matched Compound	Formula	CAS	SI	HRF Score	RSI	RHRF Score	M+ m/z	Empirical M+	M+ Lib	% Elements
97.6	2,4,6-Tris(1,1-dimethyl...	C19H32O	19687-22-0	889	99.5013	915	99.7417	276.244766	NF	Yes	100
96.6	Phenol, 2,6-bis(1,1-dimet...	C17H27NO2	1918-11-2	834	99.7551	855	99.7452	277.203630	NF	Yes	100
96.5	Phenol, 2,4,6-tris(1-meth...	C15H24O	2934-07-8	833	99.5013	836	99.498	220.182166	220.182327	Yes	100
96.1	Butylated Hydroxytoluene	C15H24O	128-37-0	814	99.5013	814	99.5013	220.182166	220.182327	Yes	100
95.6	Phenol, 4,6-di(1,1-dimet...	C15H24O	616-55-7	787	99.5013	787	99.5013	220.182166	220.182327	Yes	100
95.2	2,6-Di-tert-butyl-4-methyl...	C17H26O2	29311-34-0	769	99.5013	769	99.5013	262.192731	NF	Yes	100
94.8	Glutaric acid, 2-methylp...	C18H26O4		751	99.5013	904	100	306.182560	NF	No	100
94.6	Cyclopropanecarboxylic ...	C19H28O3	108546-75-4	741	99.5013	741	99.5013	304.203296	NF	Yes	100
94	4,6-di-tert-Butyl-m-cresol	C15H24O	497-39-2	708	99.5013	708	99.5013	220.182166	220.182327	Yes	100

图8 peak@RT11.878的NIST谱库检索结果

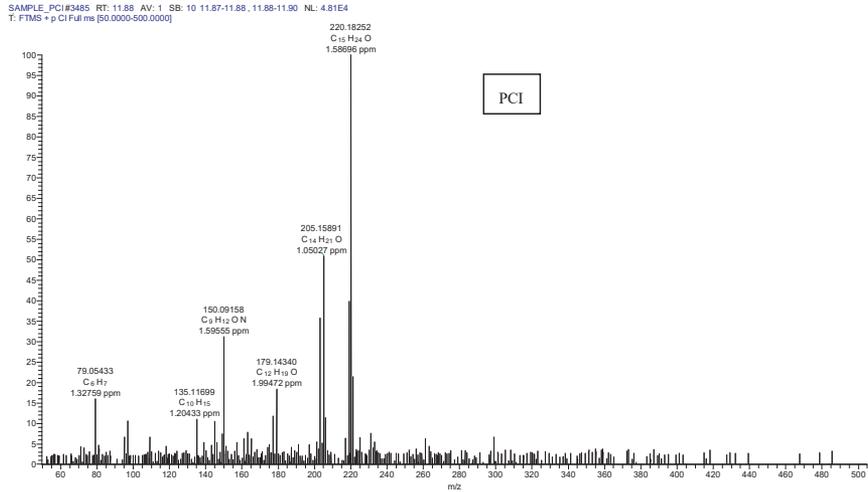
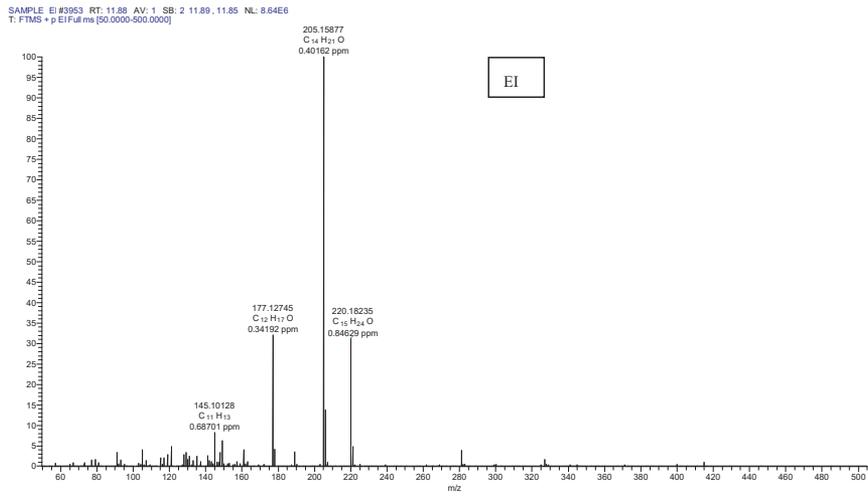


图9 peak@RT11.878的EI及PCI质谱图对比图

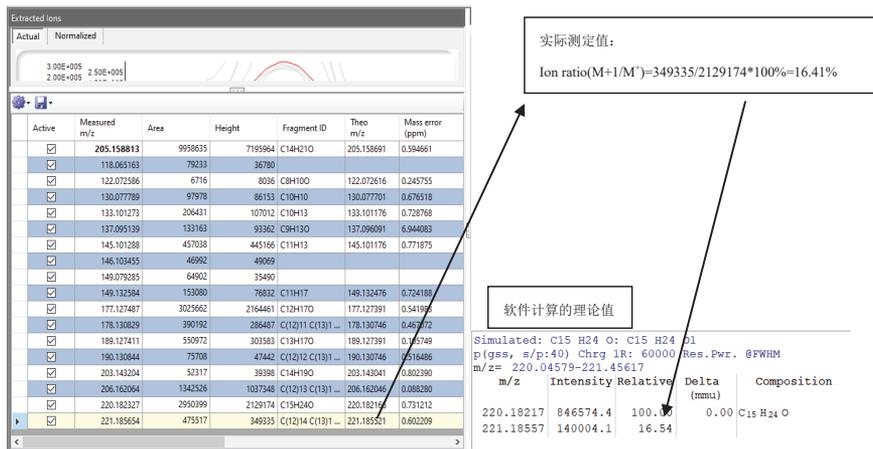


图10同位素峰丰度对比

Peak@RT12.976的定性

该峰检索结果为邻苯二甲酸二烯丙酯 (CAS#: 131-17-9)。方法与前述类似, 本文不再赘述其定性过程。

3 结论

药物中微量基因毒性物质的非靶向筛查分析具有基质干扰大、化合物种类多，含量低等分析难点。而GC Orbitrap/MS技术在全扫描分析下，能够同时实现高分辨率、高质量精度以及最低低至fg级别的灵敏度等特点。结合基毒专属谱库可同时实现上百种具有直接或潜在基因毒性物质的无偏向性筛查分析，非常适合相关行业进行药物或包材中基毒的筛查分析工作。



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