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在线全文

液相色谱-串联质谱法同时分析湖水中微囊藻毒素、柱孢藻毒素、鱼腥藻毒素和节球藻毒素的含量*

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【摘要】 **目的** 建立一种高通量、高灵敏度的液相色谱-串联质谱法，用于湖水中四类常见蓝藻毒素（微囊藻毒素、柱孢藻毒素、鱼腥藻毒素和节球藻毒素）的检测。**方法** 将水样调节为碱性后加入6种内标，用HLB与ENVI-Carb串联小柱富集，洗脱液经氮吹、复溶后上机分析。以0.1%甲酸水溶液和0.1%甲酸乙腈溶液作流动相，采用ACQUITY UPLC® BEH C_{18}（150 mm×2.1 mm，1.7 μm）色谱柱实现目标毒素的色谱分离，在多反应监测模式下采集质谱数据，内标法定量。**结果** 在一定的浓度范围内，14种蓝藻毒素呈现良好的线性关系，相关系数均大于0.998。在水样体积为100 mL的情况下，14种蓝藻毒素的方法检出限和定量限分别为0.1～0.9 ng/L和0.3～2.9 ng/L，加标回收率为81.7 %～132.9%，相对标准偏差为1.2%～14.9%。10份湖水样品中，柱孢藻毒素、鱼腥藻毒素-α和多种微囊藻毒素均有检出。**结论** 本方法具有高通量、高灵敏度、准确、可靠等特点，可用于湖水中微囊藻毒素、柱孢藻毒素、鱼腥藻毒素和节球藻毒素的同时检测。

【关键词】 蓝藻毒素 微囊藻毒素 柱孢藻毒素 液相色谱-串联质谱 湖水

Simultaneous Analysis of Microcystins, Cylindrospermopsin, Anatoxin, and Nodularin in Lake Water by Liquid Chromatography-Tandem Mass Spectrometry

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[**Abstract**] **Objective** To establish a method for simultaneous determination of trace levels of microcystins, cylindrospermopsin, anatoxin, and nodularin in lake water based on liquid chromatography-tandem mass spectrometry (LC-MS/MS). **Methods** After being adjusted to alkaline conditions and mixed with six internal standards, the water samples were enriched using dual HLB and ENVI-Carb cartridges. The eluates were then evaporated under nitrogen, reconstituted, and subjected to instrumental analysis. Both water and acetonitrile containing 0.1% formic acid were used as mobile phases. An ACQUITY UPLC® BEH C_{18} column (150 mm × 2.1 mm, 1.7 μm) was selected to separate the target cyanotoxins. Multiple reaction monitoring was applied for data acquisition, and quantification was accomplished using internal standard methods. **Results** Within certain concentration ranges, all 14 cyanotoxins examined in the study showed good linearity, with all correlation coefficients greater than 0.998. When the water volume was 100 mL, the limits of detection and quantification for the 14 cyanotoxins were 0.1-0.9 ng/L and 0.3-2.9 ng/L, respectively, and spiked recoveries and relative standard deviations were 81.7%-132.9% and 1.2%-14.9%, respectively. In the 10 lake water samples analyzed, cylindrospermopsin, anatoxin-α, and multiple microcystins were detected. **Conclusion** The method developed in the study has high-throughput capacity, as well as high sensitivity, accuracy, and reliability. The method can be applied in the simultaneous detection of microcystins, cylindrospermopsin, anatoxin, and nodularin in lake water.

[**Key words**] Cyanotoxins Microcystins Cylindrospermopsins Liquid chromatography-tandem mass spectrometry Lake water

蓝藻毒素是指由蓝细菌产生的一系列对人体或生态环境有毒有害的次生代谢产物，在全球淡水系统中最常被报道的蓝藻毒素有微囊藻毒素（microcystins, MCs）、柱孢藻毒素（cylindrospermopsins, CYNs）、鱼腥藻毒素（anatoxins, ATXs）和节球藻毒素（nodularins, NODs）[1]。其中，MCs因其在藻华水体中广泛存在、有急性肝毒性及环境持久性[2]等特点，成为最受关注的一类蓝藻毒素，其单一变体MC-LR不仅被列入国际癌症研究机构2B类致癌物清单，还被世界卫生组织和多个国家和地区管控。相

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比于MCs,其他三类蓝藻毒素的研究和报道相对较少,但其潜在风险不容忽视。CYNs是一类主要由拟柱孢藻产生的[3]、易溶于水的三环多肽生物碱,同样有肝毒性及免疫毒性[4],其主要变体CYN的半数致死量为200 μg/kg[5]。ATXs是由鱼腥藻产生的一类小分子生物碱,其最常见、毒性最强的变体为鱼腥藻毒素-α(ATX-α),可模仿乙酰胆碱与受体结合,对肌肉细胞产生强烈刺激,严重者可能导致死亡[6]。NODs的主要靶器官是肝脏和肾脏[7],虽然毒性比MCs弱,但比MCs更容易进入细胞内,可能潜在更大风险[8]。目前我国暂未管控这三类蓝藻毒素,但已有学者对CYN、ATX-α的限量或健康指导值进行了探索[9-10],2021年NOD也被国际癌症研究机构列入3类致癌物清单。此外,不同类别的蓝藻毒素可能共同存在于环境水体中[11-12],因此,亟须建立一个高通量、高灵敏度的方法用于环境水体中多类常见蓝藻毒素的检测。

蓝藻毒素的检测方法有酶联免疫吸附法、蛋白磷酸酶抑制法、高效液相色谱法、液相色谱-串联质谱法(liquid chromatography-tandem mass spectrometry, LC-MS/MS)等。其中,LC-MS/MS凭借其高选择性、高灵敏度的优势已被广泛用于蓝藻毒素的检测,MCs[13]、NOD[14]、ATX-α[15]、CYN[16]均有报道。然而,目前我国生活饮用水(GB/T 5750.8—2023)和地表水(GB/T 20466—2006)中蓝藻毒素的检测标准仅针对个别MC(3~5种),大部分研究也仅报告了1~2类蓝藻毒素,而无法针对这四类毒素进行同时分析。水中MCs和NOD常采用HLB固相萃取(solid phase extraction, SPE)小柱富集[13, 17],但ATX-α和CYN的亲水性较强,很难与MCs和NOD在同一根SPE小柱上富集。ZERVOU等[18]采用两根性质不同的SPE小柱串联实现了水中这四类蓝藻毒素的同时富集,提供了一种很好的思路;然而该方法存在仪器分析时间过长、多种毒素回收率和重现性差的问题。本研究拟参考该组合SPE策略,通过优化仪器条件和样品前处理条件,同时采用内标法定量,建立一个准确可靠且能同时分析湖水中MCs、NOD、ATX-α、CYN四类常见蓝藻毒素的LC-MS/MS方法。

1 材料和方法

1.1 仪器和试剂

AB SCIEX QTRAP 6500液相色谱-串联质谱仪(美国SCIEX公司);24位固相萃取装置(美国Supelco公司);N-EVAP™ 112氮吹仪(美国Organimation公司);SevenCompact pH计(瑞士梅特勒公司)。

ATX-α、ATX-$^{13}C_4$、CYN、CYN-$^{15}N_5$、NOD、11种MCs(即HilR、HtyR、LA、LF、LR、LW、LY、RR、WR、YR、D-Asp-LR)标准品均购自美国CFW公司;3种MC同位素内标MC-LR-$^{15}N_{10}$、MC-YR-$^{15}N_{10}$和MC-RR-$^{15}N_{13}$购自美国剑桥公司;亮氨酸脑啡肽(leucine-enkephalin, LEK)和Oasis® HLB固相萃取小柱(200 mg, 6 mL)购自美国Waters公司;ENVI-Carb固相萃取小柱(200 mg, 3 mL)购自美国Supelco公司;色谱级甲醇、乙腈和PVDF滤膜(0.45 μm, 47 mm)购自德国Merck公司;甲酸(色谱级)购自上海安谱实验科技股份有限公司;二氯甲烷(色谱级)购自美国Macron公司;氢氧化钠(AR级)购自广州化学试剂厂;去离子水由Milli-Q®Advantage A10纯水系统生成。

1.2 标准溶液配制

根据不同蓝藻毒素标准品的证书浓度,分别吸取适量标准品于5 mL容量瓶中,用甲醇定容,配成质量浓度为1000 μg/L的混合标准储备液,在—20 ℃下保存。

准确吸取14种蓝藻毒素混合标准储备液500 μL于5 mL容量瓶中,用甲醇定容,配成质量浓度为100 μg/L的混合标准工作液,在—20 ℃下保存。

准确吸取适量ATX-$^{13}C_4$、CYN-$^{15}N_5$、MC-LR-$^{15}N_{10}$、MC-YR-$^{15}N_{10}$、MC-RR-$^{15}N_{13}$和LEK标准溶液于5 mL容量瓶中,用甲醇定容,配成质量浓度为100 μg/L的混合内标储备液,在—20 ℃下保存。

1.3 样品前处理

取不少于120 mL湖水样品反复冻融3次(每次冻融先在—20 ℃下冷冻不少于12 h,再在35 ℃水浴下解冻)[13],用PVDF滤膜过滤后,准确量取100 mL水样,用2 mol/L氢氧化钠溶液调节pH至9~11,加入5 μL混合内标储备液后混匀。将HLB和ENVI-Carb小柱串联(HLB小柱在上),依次用6 mL二氯甲烷、6 mL甲醇和6 mL去离子水(pH=11)活化;将预处理后的水样以3~5 mL/min流速通过串联小柱,待水样全部流干,用6 mL 10%甲醇水溶液淋洗并抽干小柱。上下调转串联小柱的位置(即HLB小柱在下),用6 mL含0.5%甲酸的甲醇-二氯甲烷混合溶液(体积比为1∶1)洗脱,合并洗脱液。将洗脱液氮吹至近干,用0.5 mL 30%乙腈水溶液复溶,经超声5 min、涡旋3 min后,离心取上清液上机。

1.4 仪器分析

1.4.1 色谱条件

色谱柱为ACQUITY UPLC® BEH C_{18}色谱柱(150 mm×2.1 mm, 1.7 μm),柱温为40 ℃。流动相为含0.1%甲酸的水溶液(A相)和含0.1%甲酸的乙腈溶液(B相)。梯度洗脱程序为0~1.5 min, 5% B; 1.5~7.0 min, 5% B~90% B;

7.0 ~ 8.0 min, 90% B; 8.0 ~ 8.1 min, 90% B ~ 5 % B; 8.1 ~ 10.0 min, 5% B。流速为0.3 mL/min。进样体积为2 μL。

1.4.2 质谱条件

采用电喷雾离子源(electrospray ionization, ESI), 在正离子模式下, 用多反应监测模式(multiple reaction monitoring, MRM)采集数据, 14种蓝藻毒素及其内标的MRM参数见表1。气帘气为35 psi, 离子源温度为300 ℃, 离子喷雾电压为5 500 V, 雾化气为50 psi, 辅助气为60 psi。

2 结果和讨论

2.1 质谱条件优化

将标准品直接注入到质谱中, 分别在正、负离子模式下扫描目标毒素的母离子。结果发现, CYN在ESI^+、ESI^-模式下均可被检测到, 而MCs、NOD、ATX-α仅能在ESI^+模式下扫描到, 考虑到多类毒素同时分析, 最终在ESI^+下检测。在此模式下, CYN、NOD和ATX-α的母离子均以$[M+H]^+$形式存在, 但不同MC母离子可能以

表 1 14种蓝藻毒素及其内标的MRM参数

Table 1 Multiple reaction monitoring parameters of fourteen cyanotoxins and the internal standards

Toxin	Abbreviation	Retention time/min	Parent ion/(*m/z*)	Product ion/(*m/z*)	Cone voltage/V	Collision voltage/V
Anatoxin-α	ATX-α	2.76	166.0	130.9*	50	21
				149.0	50	17
Cylindrospermopsin	CYN	2.08	416.3	194.2*	70	48
				336.0	70	30
Nodularin	NOD	5.66	825.5	103.3*	220	155
				135.0	220	90
Microcystin HilR	MC-HilR	5.90	505.3	135.1*	50	15
				875.6	50	14
Microcystin HtyR	MC-HtyR	5.79	530.6	135.1*	20	14
				514.6	20	14
Microcystin LA	MC-LA	6.94	910.7	776.4*	35	30
				375.0	35	37
Microcystin LF	MC-LF	7.59	986.5	478*	20	36
				375.1	20	45
Microcystin LR	MC-LR	5.84	498.4	135.1*	20	14
				482.5	20	14
D-Aspartic acid-3-microcystin LR	[D-Asp3] MC-LR	5.82	491.3	135.2*	45	14
				847.6	45	14
Microcystin LW	MC-LW	7.41	513.3	135.1*	20	13
				891.4	20	12
Microcystin LY	MC-LY	6.98	502.0	135.1*	25	13
				486.0	25	11
Microcystin RR	MC-RR	5.39	520.2	135.1*	20	33
				620.4	20	37
Microcystin WR	MC-WR	5.93	534.7	134.9*	20	16
				934.4	20	16
Microcystin YR	MC-YR	5.77	523.6	135.0*	30	15
				507.5	30	15
Anatoxin-$^{13}C_4$	ATX-$^{13}C_4$	2.75	170.1	153.1*	45	19
Cylindrospermopsin-$^{15}N_5$	CYN-$^{15}N_5$	2.08	421.1	197.0*	90	47
Microcystin LR-$^{15}N_{10}$	MC-LR-$^{15}N_{10}$	5.84	503.4	135.0*	20	16
Microcystin YR-$^{15}N_{10}$	MC-YR-$^{15}N_{10}$	5.78	528.1	135.0*	30	15
Microcystin RR-$^{15}N_{13}$	MC-RR-$^{15}N_{13}$	5.39	526.2	135.0*	20	33
Leucine-enkephalin	LEK	5.03	556.3	396.8*	60	33

* Quantative ion.

$[M+H]^+$或$[M+2H]^{2+}$的形式存在。MC-LA、MC-LF仅能检测到$[M+H]^+$, MC-RR、MC-LR仅能扫描到$[M+2H]^{2+}$, 而其他7种MCs可同时扫描到$[M+H]^+$和$[M+2H]^{2+}$两种离子峰, 但$[M+2H]^{2+}$的丰度远高于$[M+H]^+$(≥10倍)。基于优势母离子, 进一步优化产物离子及相关参数, 选取灵敏度高、干扰少的产物离子作为定量离子和定性离子, 14种蓝藻毒素及6种内标物质的MRM参数见表1。

2.2 液相条件优化

以0.1%甲酸水溶液和0.1%甲酸乙腈溶液作为流动相, 比较ACQUITY UPLC® BEH C_{18}色谱柱(150 mm×2.1 mm, 1.7 μm)、ZIC-HILIC色谱柱(150 mm×2.1 mm, 3.5 μm)、TSKgel Amide-80色谱柱(150 mm×2.1 mm, 3 μm)对目标毒素的保留和分离特性。结果表明, ZIC-HILIC柱和Amide-80柱对ATX-α和CYN保留较好, 但无法有效分离各MC变体和NOD, 峰型也很差; 而BEH C_{18}柱可实现11种MCs和NOD的保留和分离, 但对ATX-α和CYN的保留较弱, 且存在溶剂效应(双峰)。进一步优化发现, 当初始有机相比例降低至5%时, ATX-α和CYN在BEH C_{18}柱上的保留得到增强, 且溶剂效应消失。因此, 选择ACQUITY UPLC® BEH C_{18}色谱柱作为分析柱, 且优化后的梯度可在10 min内实现14种目标蓝藻毒素的分析。

2.3 水样前处理条件优化

考虑到目标蓝藻毒素极性差异较大且多数具有平面结构, 本研究测试了广谱型SPE小柱HLB和两款石墨碳SPE小柱(ENVI-Carb和HyperSep™ PGC)对14种目标毒素的富集能力。结果发现, HLB小柱对所有MCs和NOD都有较好的保留能力, 且对ATX-α有一定的保留, 但无法保留CYN; 而两种石墨碳小柱对四类蓝藻毒素均有一定的保留, 但个别MC无保留。相比于PGC小柱, ENVI-Carb小柱对CYN保留能力更好。因此, 后续基于HLB和ENVI-Carb串联小柱对水样pH值、淋洗溶剂、洗脱溶剂等条件进行优化。

2.3.1 水样pH值

比较不同pH(3、7、9、11)的水样通过串联小柱时目标蓝藻毒素的回收情况, 见图1。ATX-α在酸性条件下无法保留, 在中性和碱性条件下保留能力较好(59%~70%); 而MCs、NOD和CYN均在碱性条件下回收最好(58%~107%), 显著高于在酸性和中性条件下(0%~81%)。最终选择将水样调节至pH为9~11时上样。

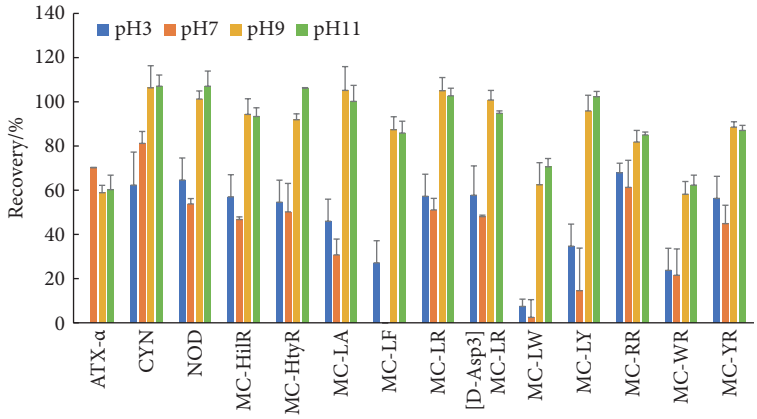

图 1 不同pH值水样对14种蓝藻毒素的回收率

Fig 1 Recoveries of fourteen cyanotoxins from water samples with different pH values

The abbreviations are explained in Table 1.

2.3.2 淋洗溶剂

比较不淋洗、纯水淋洗和10%甲醇水溶液淋洗HLB + ENVI-Carb串联小柱时目标蓝藻毒素的回收情况。结果发现三种淋洗溶剂条件下14种蓝藻毒素的回收无显著差异, 考虑到淋洗溶剂中甲醇比例越高, 对有机杂质去除效果越好, 最终选择10%甲醇水溶液为淋洗溶剂。

2.3.3 洗脱溶剂

比较纯甲醇、含0.1%甲酸的甲醇、含0.5 %甲酸的甲醇、含0.5 %甲酸的甲醇-二氯甲烷混合溶液(体积比为1∶1)对串联小柱上目标蓝藻毒素的洗脱能力, 结果见图2。纯甲醇对MCs和NOD具有较好的洗脱效果(66%~93%), 但对ATX-α(28%)和CYN(0%)的洗脱能力弱; 在甲醇中添加0.5%的甲酸可增加ATX-α (55%)和CYN(36%)的洗脱; 再增加50%二氯甲烷后, 可将CYN完全洗脱下来, 且其他毒素的洗脱也有所增强。因此, 最终选择6 mL含0.5%甲酸的甲醇-二氯甲烷混合溶液(体积比为1∶1)作为洗脱溶剂。

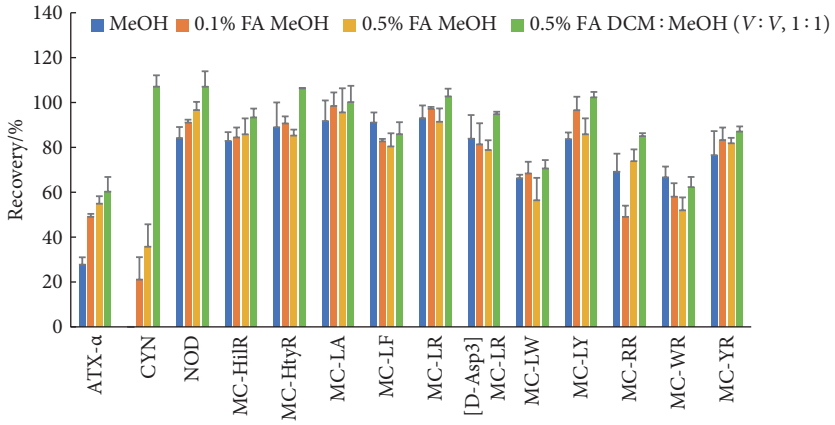

图 2 不同洗脱溶剂下14种蓝藻毒素的回收率

Fig 2 Recoveries of fourteen cyanotoxins under different elution solvents

MEOH: methanol; 0.1% FA MEOH: methanol containing 0.1% formic acid; 0.5% FA MEOH: methanol containing 0.5% formic acid; 0.5% FA DCM ∶ MeOH: dichloromethane and methanol mixture containing 0.5% formic acid; the other abbreviations are explained in Table 1.

2.3.4 复溶溶剂

比较5%、10%、20%、30%、40%乙腈水溶液对洗脱液氮吹后的复溶效率。结果表明, 发现5%乙腈水溶液不足以有效复溶大部分MCs(10% ~ 68%); 当乙腈比例增加到30%时, 所有目标蓝藻毒素均可完全复溶。同时, 为降低复溶液和初始流动相的有机相比例不同导致的溶剂效应, 本研究将进样体积从10 μL调整为2 μL, 此时, 色谱峰型不受影响, 溶剂效应消失。

2.3.5 水样浓缩倍数

比较加标真实湖水样本浓缩50、200、1 000倍时14种蓝藻毒素的回收情况。结果发现, 当浓缩倍数为1 000时, MC-RR回收率和稳定性明显变差, 噪声明显增加, 信噪比显著降低; 而浓缩倍数为50和200时, 回收率正常。其他13种蓝藻毒素在不同浓缩倍数下均能获得较好回收。考虑到更低的检出限, 最终选择将水样浓缩200倍。

2.4 方法性能评价

2.4.1 标准曲线的线性

在0.05 ~ 150 μg/L范围, 配制一组含有8个浓度的系列标准溶液, 所有标准溶液中内标质量浓度均为1 μg/L。按照相似结构、相近回收率和保留时间等原则, 在6个备选内标中选择合适的内标物质(表2)。以目标毒素的浓度为横坐标, 以目标毒素和相应内标的峰面积比值为纵坐标, 绘制14种蓝藻毒素的标准曲线。如表2所示, 在一定的浓度范围内, 14种目标蓝藻毒素呈现良好的线性, 相关系数均大于0.998。

2.4.2 回收率和精密度

取不含待测毒素的湖水样品进行加标回收实验, 设置3个加标水平(2.5 ng/L、10 ng/L、25 ng/L), 每个水平做

表 2 14种蓝藻毒素的线性方程和相关系数

Table 2 Linearity equations and coefficients of 14 cyanotoxins

Toxin	Concentration range/(μg/L)	Linear equation	Coefficient	Internal standard
ATX-α	0.5-150	$y = 0.128x - 0.012$	0.9997	ANA-$^{13}C_4$
CYN	0.05-150	$y = 0.477x - 0.002$	0.9991	CYN-$^{15}N_5$
NOD	0.5-150	$y = 0.105x - 0.034$	0.9993	LEK
MC-HilR	0.1-150	$y = 0.842x - 0.006$	0.9982	MC-YR-$^{15}N_{10}$
MC-HtyR	0.1-150	$y = 1.012x + 0.050$	0.9983	MC-YR-$^{15}N_{10}$
MC-LA	0.5-150	$y = 0.409x + 0.004$	0.9997	LEK
MC-LF	0.1-150	$y = 0.129x - 0.007$	0.9996	LEK
MC-LR	0.1-150	$y = 0.407x + 0.035$	0.9992	MC-LR-$^{15}N_{10}$
[D-Asp3] MC-LR	0.1-150	$y = 0.419x + 0.006$	0.9992	MC-LR-$^{15}N_{10}$
MC-LW	0.1-150	$y = 0.364x - 0.0100$	0.9994	MC-RR-$^{15}N_{13}$
MC-LY	0.5-150	$y = 0.116x + 0.0004$	0.9985	MC-LR-$^{15}N_{10}$
MC-RR	0.5-150	$y = 0.541x - 0.049$	0.9989	MC-RR-$^{15}N_{13}$
MC-WR	0.5-150	$y = 0.502x - 0.039$	0.9995	MC-RR-$^{15}N_{13}$
MC-YR	0.1-150	$y = 0.783x + 0.017$	0.9988	MC-YR-$^{15}N_{10}$

The abbreviations are explained in Table 1.

6个平行。ATX-α、CYN、NOD和MCs的回收率分别为89.8% ~ 103.0%、85.6% ~ 100.3%、124.1% ~ 132.9%和81.7% ~ 117.3%，相对标准偏差(relative standard deviation, RSD)分别为2.4 % ~ 6.5 %、3.0% ~ 7.2%、3.3% ~ 11.6%、1.2% ~ 14.9%(表3)。与外标法相比，内标法显著提高了ATX-α和CYN的定量准确性，由原来的57.4%和69.5%上升到89.8%和85.6%；而其他蓝藻毒素定量的准确性也有一定增强。

2.4.3 检出限和定量限

在低浓度加标水样中，按3∶1和10∶1的信噪比分别计算14种蓝藻毒素的方法检出限(limit of detection, LOD)和定量限(limit of quantitation, LOQ)。如表3所示，水中11种MCs、CYN、ATX-α和NOD的方法检出限依次为0.1 ~ 0.9 ng/L、0.1 ng/L、0.7 ng/L和0.7 ng/L，方法定量限依次为0.3 ~ 2.9 ng/L、0.4 ng/L、2.5 ng/L和2.3 ng/L。

2.5 方法应用

将优化后的方法用于10份湖水样品中，结果见表4。除NOD、MC-HtyR外，其他12种蓝藻毒素至少在1份水样中检出，检出率为10% ~ 100%。其中，CYN在所有水样中均检出，平均值和中位数分别为109.9 ng/L和98.9 ng/L，明显高于ATX-α和MCs。ATX-α也在4份水样中检出，质量浓度为< 0.7 ~ 29.6 ng/L，高于大多数单一MC。MCs中，

表 3 3种加标水平下湖水中14种蓝藻毒素的回收率、检出限和定量限

Table 3 Recovery, limits of detection (LOD), and limits of quantification (LOQ) of 14 cyanotoxins in lake water samples at 3 spiked levels

Toxin	Spiked level						LOD/(ng/L)	LOQ/(ng/L)
	2.5 ng/L		10 ng/L		25 ng/L			
	Recovery/%	RSD/%	Recovery/%	RSD/%	Recovery/%	RSD/%		
ATX-α	100.4	6.5	89.8	3.9	103.0	2.4	0.7	2.5
CYN	95.4	7.2	85.6	3.0	100.3	3.6	0.1	0.4
NOD	124.1	8.5	132.9	11.6	132.2	3.3	0.7	2.3
MC-HilR	108.2	8.0	116.6	6.3	92.9	1.2	0.4	1.5
MC-HtyR	109.3	7.2	111.7	8.7	95.4	9.3	0.3	1.0
MC-LA	106.8	4.9	101.6	5.4	109.3	2.9	0.8	2.6
MC-LF	89.3	5.3	100.0	6.3	90.0	7.9	0.2	0.6
MC-LR	92.4	8.5	94.8	8.0	106.6	4.2	0.1	0.3
[D-Asp3] MC-LR	94.8	6.5	93.0	14.9	102.9	5.1	0.3	0.9
MC-LW	94.4	10.3	92.8	6.8	93.1	7.6	0.2	0.7
MC-LY	106.1	11.2	106.3	8.1	116.8	9.1	0.8	2.6
MC-RR	90.9	9.8	95.6	11.3	95.6	2.6	0.6	2.0
MC-WR	84.9	7.2	81.7	3.3	88.3	8.5	0.9	2.9
MC-YR	110.2	6.4	117.3	5.7	110.7	8.2	0.1	0.3

RSD: relative standard deviation; LOD: limit of detection; LOQ: limit of quantitation; the other abbreviations are explained in Table 1.

表 4 10份湖水样品中14种蓝藻毒素的检出情况

Table 4 Detection of 14 cyanotoxins in 10 lake water samples

Toxin	Detection number	Detection rate/%	Mean/(ng/L)	Median/(ng/L)	P_{95}/(ng/L)	Range/(ng/L)
ATX-α	4	40	3.9	–	18.1	N.D.-29.6
CYN	10	100	109.9	98.9	266.3	1.9-322.2
NOD	0	0	–	–	–	N.D.
MC-HilR	3	30	0.5	–	1.4	N.D.-1.5
MC-HtyR	0	0	–	–	–	N.D.
MC-LA	8	80	1.5	1.3	2.5	N.D.-2.6
MC-LF	1	10	0.3	–	0.9	N.D.-1.7
MC-LR	6	60	12.4	4.8	49.7	N.D.-64.4
[D-Asp3] MC-LR	6	60	6.2	1.9	20.5	N.D.-21.1
MC-LW	4	40	0.4	–	1.5	N.D.-2.2
MC-LY	3	30	2.3	–	9.8	N.D.-14
MC-RR	5	50	9.3	1.5	32.6	N.D.-40.1
MC-WR	2	20	2.2	–	10.0	N.D.-17.3
MC-YR	3	30	1.0	–	4.4	N.D.-6.4
∑MCs	/	/	36.3	11.2	121.9	2.4-153.8

N.D.: not detected; ∑MCs: the total concentration of 11 MCs, with the levels below the limit of detection (LOD) substituted by LOD/2; the other abbreviations are explained in Table 1. Some results are represented by –, which indicates that the statistical result is below the LOD.

MC-LR、MC-RR和[D-Asp3] MC-LR的平均浓度最高, 分别为12.4 ng/L、9.3 ng/L和6.2 ng/L。尽管单一MC含量较低, 但考虑到多种MCs相似的毒性[19-20], 总MCs质量浓度可达2.4 ~ 153.8 ng/L。可见, 地表水中往往存在多种类别的毒素, 仅监测个别MC可能低估蓝藻毒素带来的总体风险。标准溶液和水样中目标蓝藻毒素的色谱图见图3。

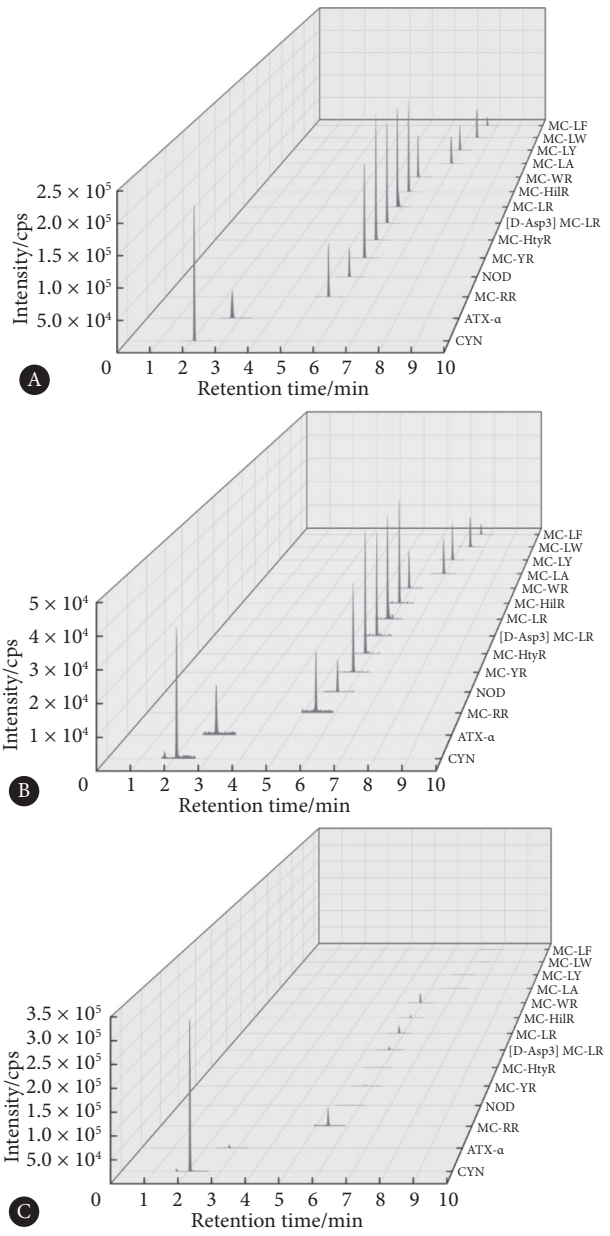

图 3 14种蓝藻毒素在不同样品中的色谱图

Fig 3 The chromatograms of 14 cyanotoxins in different samples

The abbreviations are explained in Table 1. A, 10 μg/L standard solution; B, 10 ng/L spiked lake water sample; C, a real lake water sample.

3 小结

本研究基于组合式SPE小柱和LC-MS/MS技术建立了一种湖水中MCs、CYN、ATX-α、NOD四类常见蓝藻毒素的同时分析方法。与以往的多毒素方法[18]相比, 该方法一次进样仅需10 min, 大大缩短了仪器分析时间(40 min); 通过优化SPE小柱的类型、水样上样条件、淋洗洗脱条件、浓缩倍数等条件, 同时应用内标法定量, 大幅提升了方法的准确性和稳定性。该方法具有高通量、高灵敏度、准确、可靠等优点, 可用于湖水中这四类蓝藻毒素的痕量检测。

* * *

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