

# 液液萃取 - 高效液相色谱串联质谱测定尿中的九种邻苯二甲酸单酯

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**摘要:**目的 建立同时测定尿中九种常见邻苯二甲酸酯代谢物邻苯二甲酸单酯(monoester phthalate metabolites, mPAEs)的液液萃取-超高效液相色谱串联三重四极杆质谱方法。方法 1 ml 尿样加入乙酸铵水溶液和 $\beta$ -葡萄糖醛酸酶于37℃摇床酶解16 h。加入20  $\mu$ l 甲酸涡旋混匀,再加入3 ml 乙酸乙酯涡旋5 min 进行液液萃取,离心后取上清液氮吹浓缩,经0.20 ml 乙腈-水溶液(50:50, v/v)复溶后,用BEH C<sub>18</sub>色谱柱(2.1 mm  $\times$  100 mm, 1.7  $\mu$ m)分离,流动相为0.10% 乙酸乙腈和0.10% 乙酸水溶液,采用电喷雾负离子模式电离和多反应监测模式质谱检测,内标标准曲线定量。结果 邻苯二甲酸酯类在0.50~200 ng/ml 范围内有良好线性关系,相关系数大于0.999。当取1.0 ml 尿液用于测定时,方法检出限为 $1.5 \times 10^{-3}$ ~0.29 ng/ml,定量限为 $4.9 \times 10^{-3}$ ~0.98 ng/ml。加标回收率为87.05%~111.5%,日内和日间相对标准偏差分别为0.31%~3.05%和0.59%~3.46%。采用建立的方法测定了正常人尿样277例,除邻苯二甲酸单苄酯(mono-benzyl phthalate, MBzP)的检出率为87.73%以外,其余8种mPAEs的检出率均为100%。其中邻苯二甲酸单丁酯(Mono-butyl phthalate, MnBP)检出含量最高,中位数水平为194.10  $\mu$ g/g<sub>肌酐</sub>; MBzP含量最低,中位数水平为 $3.7 \times 10^{-2}$   $\mu$ g/g<sub>肌酐</sub>。结论 建立了尿样中9种邻苯二甲酸单酯的超高效液相色谱串联质谱同时检测方法,可实现其灵敏、准确检测,为其暴露评估提供技术支持。

**关键词:**尿样;邻苯二甲酸单酯;超高效液相色谱-串联三重四极杆质谱法;液液萃取法

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## Determination of nine monophthalates in urine by liquid-liquid extraction - high performance liquid chromatography - tandem mass spectrometry

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**Abstract: Objective** To develop a liquid-liquid extraction combined with ultra-high performance liquid chromatography-tandem triple quadrupole mass spectrometry (LLE-UHPLC-MS/MS) method for simultaneous determination of nine monoester phthalate metabolites (mPAEs) in urine. **Methods** 1 ml urine sample was subjected to enzymatic hydrolysis by the addition of ammonium acetate solution and  $\beta$ -glucuronidase at 37 °C on a shaker for 16 h. Subsequently, 20  $\mu$ l of formic acid was added and thoroughly mixed. Liquid-liquid extraction was performed with 3 ml of ethyl acetate, then the mixture was centrifuged and the supernatant was concentrated under nitrogen flow. The residue was reconstituted with 0.20 ml of acetonitrile-water solution (50:50, v/v). Chromatographic separation was performed on a BEH C<sub>18</sub> column (2.1 mm  $\times$  100 mm, 1.7  $\mu$ m) using a mobile phase of 0.10% acetic acid in acetonitrile and 0.10% aqueous acetic acid. Analytes were ionized via electrospray ionization (ESI) in negative ion mode and detected by multiple reaction monitoring (MRM), and quantification was achieved through the internal standard curves. **Results** Good linear relationship was obtained for the analytes within 0.50 to 200 ng/ml, with correlation coefficients greater than 0.999. When 1 ml of urine sample was utilized for analysis, the detection limits and the quantitation limits of the method were  $1.5 \times 10^{-3}$  to 0.29 ng/ml and  $4.9 \times 10^{-3}$  to 0.98 ng/ml, respectively. The recovery rates of the method ranged from 87.05% to 111.5%, with intra-day relative standard deviations of 0.31% to 3.05%, and inter-day relative standard deviations of 0.59% to 3.46%. The nine monophthalates in 277 urine

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samples from healthy individuals were determined by the proposed method. Except for mono-benzyl phthalate (MBzP), which had a detection rate of 87.73%, the detection rates of other monophthalates were 100%. Mono-butyl phthalate (MnBP) had the highest median concentration of 194.10  $\mu\text{g/g}$  creatinine, while MBzP had the lowest median concentration of  $3.7 \times 10^{-2}$   $\mu\text{g/g}$  creatinine. **Conclusion** The established method for simultaneous determination of 9 monophthalates in urine samples was simple, sensitive, and accurate, which is suitable for the accurate exposure assessment of these substances in urine.

**Keywords:** Urine; Monophthalates; Ultra-high performance liquid chromatography-tandem triple quadrupole mass spectrometry; Liquid-liquid extraction

邻苯二甲酸酯(phthalate esters, PAEs)是一类具有一个苯环、两个羰基和两个醇基的二酯结构的物质,通常呈无色油状。PAEs 是使用最广泛的增塑剂,占塑化剂总用量的 70% 左右。其易挥发和浸出的特性使其成为环境主要污染物<sup>[1]</sup>,并可通过饮食、空气、化妆品等介质进入人体<sup>[2-3]</sup>,对人类健康造成潜在不良影响<sup>[4-5]</sup>。目前已在尿液、血液、母乳和精液等生物样本中检测出多种 PAEs 代谢物——邻苯二甲酸单酯(mPAEs)<sup>[6]</sup>。美国环境保护署(EPA)已将邻苯二甲酸二甲酯(dimethyl phthalate, DMP)、邻苯二甲酸二乙酯(diethyl phthalate, DEP)、邻苯二甲酸苄丁酯(benzyl butyl phthalate, BBP)、邻苯二甲酸二正丁酯(di-n-butyl phthalate, DnBP)、邻苯二甲酸二正辛酯(di-n-octyl phthalate, DnOP)和邻苯二甲酸二(2-乙基己基)酯(di-2-ethylhexyl phthalate, DEHP)列为优先控制污染物<sup>[7]</sup>。研究表明,低分子量的 PAEs 的毒性高于高分子量的邻苯二甲酸酯<sup>[8]</sup>。

因邻苯二甲酸酯污染广泛,评估其暴露水平至关重要。对于 PAEs,常用尿液中的 mPAEs 浓度来反映暴露水平。在测定尿中 mPAEs 时,气相色谱-质谱法(GC-MS)<sup>[9-13]</sup>和高效液相色谱-串联质谱法(HPLC-MS)<sup>[14-18]</sup>均有应用,但鉴于大多邻苯二甲酸单酯化合物的沸点较高,气相色谱分析时须进行衍生,耗时繁琐,且易引入分析误差。因此,HPLC-MS 是测定 mPAEs 的首选技术。mPAEs 分析时,常采用固相萃取(SPE)进行样品净化,因其有高且稳定的回收率<sup>[19-21]</sup>。但笔者发现 SPE 耗时且经济成本较高。液液萃取(LLE)是一种简单、快速、低成本的萃取方法,在污染物分析中被广泛使用,但在尿中 mPAEs 检测中多限于 DEHP<sup>[22-25]</sup>。故本研究采用 LLE 样品前处理,建立了尿中 9 种 mPAEs 的超高效液相色谱-串联质谱方法,并成功用于实际样品分析。

## 1 材料与方法

### 1.1 仪器与试剂

**1.1.1 仪器** 超高效液相色谱-串联三重四极杆质谱仪(Waters ACQUITY UPLC I-Class / Xevo TQ-XS, 美国); ACQUITY UPLC<sup>®</sup> BEH C<sub>18</sub> 色谱柱(2.1 mm  $\times$  100 mm, 1.7  $\mu\text{m}$ , Waters, 美国); ACQUITY UPLC

BEH C<sub>18</sub> VanGuard 保护柱(Waters, 美国); 冷冻离心机(eppendorf 5810R, 德国); 高通量全自动平行浓缩仪(睿科 Auto EVA 80, 中国); -80  $^{\circ}\text{C}$  超低温冷冻储存箱(美菱 DW-HL680, 中国)。

**1.1.2 标准品** 邻苯二甲酸单乙酯(Monoethyl Phthalate, MEP)、邻苯二甲酸单(2-乙基己基)酯(Mono(2-ethylhexyl) Phthalate, MEHP)、邻苯二甲酸单苄酯(Monobenzyl Phthalate, MBzP)、邻苯二甲酸单正丁酯(Mono-n-butyl Phthalate, MnBP)、邻苯二甲酸单异丁酯(Mono-iso-butyl Phthalate, MiBP)标准品购买于 AccuStandard 公司,邻苯二甲酸单(2-乙基-5-氧代己基)酯(Mono(2-ethyl-5-oxohexyl) Phthalate, MEOHP)、邻苯二甲酸单(2-羧基甲基己基)酯(Mono(2-carboxymethylhexyl) Phthalate, MCMHP)、邻苯二甲酸单(2-乙基-5-羧基戊基)酯(Mono(2-ethyl-5-carboxypentyl) Phthalate, MECPP)、邻苯二甲酸单(2-乙基-5-羟基己基)酯(Mono(2-ethyl-5-hydroxyhexyl) Phthalate, MEHHP)标准品购买于 Toronto research chemicals 公司。碳-13 标记的邻苯二甲酸单(2-乙基己基)酯(MEHP-<sup>13</sup>C<sub>4</sub>)、碳-13 标记的邻苯二甲酸单(2-乙基-5-羟基己基)酯(MEHHP-<sup>13</sup>C<sub>4</sub>)、碳-13 标记的邻苯二甲酸单(2-乙基-5-氧代己基)酯(MEOHP-<sup>13</sup>C<sub>4</sub>)、碳-13 标记的邻苯二甲酸单(2-乙基-5-羧基戊基)酯(MECPP-<sup>13</sup>C<sub>4</sub>)、碳-13 标记的邻苯二甲酸单(2-羧基甲基己基)酯(MCMHP-<sup>13</sup>C<sub>4</sub>)、碳-13 标记的邻苯二甲酸单乙酯(MEP-<sup>13</sup>C<sub>4</sub>)、碳-13 标记的邻苯二甲酸单苄酯(MBzP-<sup>13</sup>C<sub>4</sub>)、碳-13 标记的邻苯二甲酸单异丁酯(MiBP-<sup>13</sup>C<sub>4</sub>)、碳-13 标记的邻苯二甲酸单正丁酯(MnBP-<sup>13</sup>C<sub>4</sub>)标准品均购买于 Cambridge isotope laboratories 公司。

**1.1.3 试剂** 甲醇(LC-MS, Thermo Fisher Scientific 公司, 美国); 乙酸铵(GR, 98%, 科隆, 中国); 乙酸乙酯(HPLC, Thermo Fisher Scientific 公司, 美国); 二氯甲烷(HPLC, 科密欧); 乙腈(LC-MS, Thermo Fisher Scientific, 美国); 氨水(LC-MS,  $\geq 25\%$ , 阿拉丁, 中国); 甲酸(色谱级,  $\geq 98\%$ , 阿拉丁, 中国); 乙酸(LC-MS, Thermo Fisher Scientific, 美国);  $\beta$ -葡萄糖醛

酸酶/芳基硫酸酯酶( $\beta$ -葡萄糖醛酸苷酶活性 > 100 000 units/ml, 硫酸酯酶活性 < 20 000 units/ml) (Sigma - aldrich, 美国)。

**1.1.4 标准溶液的制备** 分别准确称取各标准品一定量于 10 ml 容量瓶中,以甲醇(质谱级)溶解并定容,分别制成浓度为 1.00 mg/ml 的标准储备溶液。采用质谱级甲醇将配制好的标准储备液稀释至 1.00  $\mu$ g/ml 作为混合标准应用液。采用质谱级甲醇溶解 9 种邻苯二甲酸单酯类物质内标标准品并稀释至 100  $\mu$ g/ml 作为内标标准储备溶液。分别准确吸取配制好的内标标准储备溶液,用甲醇释至 1.00  $\mu$ g/ml,作为混合内标标准应用液。所有标准储备液及标准应用液放置于 -20  $^{\circ}$ C 冰箱避光保存。

**1.2 实验方法**

**1.2.1 尿液采集与保存** 尿液样品均来自华西医院健康体检人群。采用 15 ml 或 50 ml 聚丙烯离心管收集尿液,冷链运输至实验室,置于 -80  $^{\circ}$ C 低温冰箱保存。同时用纯水做实验空白。

**1.2.2 色谱条件** 分离柱为 ACQUITY UPLC BEH  $C_{18}$  色谱柱(2.1 mm  $\times$  100 mm, 1.7  $\mu$ m, 美国 Waters)。

流动相为 0.1% 乙酸水溶液(流动相 A)和 0.1% 乙酸-乙腈溶液(流动相 B),梯度洗脱程序见表 1。流速为 0.30 ml/min,柱温为 40 $^{\circ}$ C,自动进样器温度为 10  $^{\circ}$ C。进样体积为 5  $\mu$ l。表 1 为液相色谱梯度洗脱程序。

**1.2.3 质谱条件** 采用电喷雾电离源(ESI)负离子模式和多反应监测模式(MRM)检测。电离源温度为 150 $^{\circ}$ C,毛细管电压为 2.5 kV,锥孔电压为 26 V,脱溶剂气、锥孔气、雾化气流量分别为 1 000 L/h、150 L/h、7 Ba,碰撞气流速为 0.15 ml/min,脱溶剂气温度为 500  $^{\circ}$ C。表 2 为保留时间和质谱参数。

表 1 液相色谱梯度洗脱程序

Table 1 Gradient elution program for HPLC

时间(min)	流动相 A(%)	流动相 B(%)
0.00	70	30
4.00	70	30
9.00	5.00	95
10.00	5.00	95
10.10	70	30
15.00	70	30

表 2 被测物的保留时间和质谱参数

Table 2 The retention times and mass spectrometric parameters of the analytes

被测物	保留时间(min)	母离子(m/z)	子离子(m/z)	驻留时间(s)	锥孔电压(V)	碰撞能量(eV)	分段检测时间(min)
MEP	2.22	193.03	76.97 <sup>a</sup> 120.94	0.22 $\times 10^{-1}$	6.0	14 10	0~4
MEP- <sup>13</sup> C <sub>4</sub>	2.22	197.03	78.98 <sup>a</sup> 123.20	0.22 $\times 10^{-1}$	12	18 8	0~4
MECPP	5.62	307.03	159.02 <sup>a</sup> 113.05	0.50 $\times 10^{-2}$	2.0	10 28	5~8
MECPP- <sup>13</sup> C <sub>4</sub>	5.62	311.03	159.01 <sup>a</sup> 113.05	0.50 $\times 10^{-2}$	24	10 32	5~8
MEHHP	6.13	293.03	145.04 <sup>a</sup> 120.92	0.50 $\times 10^{-2}$	2.0	12 18	5~7.5
MEHHP- <sup>13</sup> C <sub>4</sub>	6.15	296.97	123.98 <sup>a</sup> 78.98 144.99	0.50 $\times 10^{-2}$	6.0	20 30 14	5~7.5
MiBP	6.23	221.10	76.97 <sup>a</sup> 134.00 70.98	0.50 $\times 10^{-2}$	20	16 14 14	4~7
MiBP- <sup>13</sup> C <sub>4</sub>	6.20	225.10	78.98 <sup>a</sup> 136.98	0.50 $\times 10^{-2}$	20	16 14	4~7
MEOHP	6.33	291.03	143.03 <sup>a</sup> 120.92	0.50 $\times 10^{-2}$	6.0	12 18	5~7.5
MEOHP- <sup>13</sup> C <sub>4</sub>	6.33	295.10	143.02 <sup>a</sup> 123.97 78.98	0.50 $\times 10^{-2}$	22	12 16 30	5~7.5
MNBP	6.41	221.10	76.97 <sup>a</sup> 70.98	0.50 $\times 10^{-2}$	10	16 12	4~7
MNBP- <sup>13</sup> C <sub>4</sub>	6.40	225.10	78.98 <sup>a</sup> 151.02	0.50 $\times 10^{-2}$	10	16 12	4~7
MCMHP	6.62	307.03	159.02 <sup>a</sup> 112.99	2.50 $\times 10^{-2}$	20	12 24	4~7

(续表)

被测物	保留时间 (min)	母离子 (m/z)	子离子 (m/z)	驻留时间 (s)	锥孔电压 (V)	碰撞能量 (eV)	分段检测时间 (min)
MCMHP-13C <sub>4</sub>	6.62	311.03	159.01 <sup>a</sup> 113.05	0.50 × 10 <sup>-2</sup>	20	14 26	4~7
MBZP	6.71	255.10	76.97 <sup>a</sup> 107.33	0.50 × 10 <sup>-2</sup>	2.0	18 14	5~7.5
MBZP- <sup>13</sup> C <sub>4</sub>	6.71	259.03	107.00 <sup>a</sup> 79.43	0.50 × 10 <sup>-2</sup>	24	12 16	5~7.5
MEHP	8.79	276.97	133.98 <sup>a</sup> 127.06	3.50 × 10 <sup>-2</sup>	2.0	14 14	7~9.5
MEHP- <sup>13</sup> C <sub>4</sub>	8.79	281.03	136.98 <sup>a</sup> 78.92	3.50 × 10 <sup>-2</sup>	16	16 16	7~9.5

注:a 定量离子。

**1.2.4 标准曲线绘制** 用乙腈-水溶液(50:50, v/v)将混合标准溶液稀释至浓度分别为 0.50、1.00、5.00、10.00、20.00、50.00、100 和 200 ng/ml, 内标浓度均为 20.00 ng/ml。以 1/x 为权重, 以横坐标为浓度, 纵坐标为待测物峰面积与同位素内标峰面积之比作标准曲线。

**1.2.5 样品分析** 将冻存的尿样取出至室温解冻, 涡旋混匀后移取 1.00ml, 加入 200 μl 1 mol/L 乙酸铵溶液(pH 6.0)、40 μl 100 ng/ml 混合内标标准应用液和 20 μl β-葡萄糖醛酸酶, 充分涡旋混匀后, 置于 37℃ 摇床酶解 16 h。酶解完成后, 取出冷却至室温,

$$\text{样品含量}(\mu\text{g/g}_{\text{肌酐}}) = \frac{\text{根据标准曲线计算得到的样液含量}(\text{ng/ml}) \times 0.20 \times 3.00}{1.00 \times 2.50 \times \text{尿肌酐含量}(\text{g/L})}$$

**1.2.6 质量控制** 参考 ICH《M10: 生物分析方法验证及样品分析》指导原则制定质量控制程序<sup>[26]</sup>。每批次样品包括程序空白、试剂空白、混合标准溶液、加标回收样以及平行试验。试剂空白为每次实验所用的复溶液 50% 乙腈水溶液, 为了解 50% 乙腈水溶液和仪器系统所带来的污染情况, 在进样序列开始时首先上机检测, 若发现存在污染, 需寻找原因并排除, 将污染所导致的背景值降至方法检出限以下。程序空白为前处理过程中, 除不加尿液样外, 其他步骤同“1.2.5 样品分析”一致, 用于监测整个实验阶段所使用的试剂、耗材和仪器带来的干扰, 若检出待测物, 则尿液样的检出浓度需扣除程序空白。

20 个样品检测后插入标准溶液复测, 标准溶液各浓度点的检测值应在理论值范围内(一般为 ±15% 以内, 最低浓度在 ±20% 以内)。加标回收率超出 70%~120% 范围, 需查找原因后重新分析。平行试验采用对同一样品进行重复分析, 两次检测结果的差值应不超过平均值的 20%, 否则须重新分析。

**1.2.7 数据处理** 采用 excel 软件对尿样中 mPAEs 的检出水平进行统计描述, 检出率高于 75% 的目标

加入 20 μl 甲酸再次涡旋混匀。加入 3.00 ml 乙酸乙酯并涡旋 5 min 进行液液萃取, 以 1 274 g 的速度离心 5 min, 取上清液 2.50 ml, 40℃ 水浴氮吹浓缩至近干, 加入 200 μl 50% 乙腈复溶, 涡旋混匀后再以 29 703 g 离心 5 min, 取上清液用于进样分析。尿样中 mPAEs 的实际含量为测定值扣除空白, 为避免出汗和饮水导致尿液浓缩或稀释, 从而影响暴露水平评估, 采用尿肌酐的含量对尿液目标化合物含量进行了校正。尿样中肌酐的检测参考 WS/T 98-1996 高效液相色谱法测定。样品中待测物含量计算公式如下:

化合物浓度以中位数、上四分位数和下四分位数表示。

## 2 结果与讨论

**2.1 质谱条件优化** 将 100 ng/ml 的各化合物标准溶液分别注入 Waters Xevo TQ-XS 质谱仪流动注射泵, 在 combine 模式下以 50 μl/min 注射流速注射, 流动相为 70% 甲醇水溶液, ESI 负离子模式电离检测。在全扫描模式下扫描出 [M-H]<sup>-</sup> 离子, 确定前体离子后进行 MRM 产物离子扫描优化, 得到各待测物质谱参数。按照“1.2.2 色谱条件”分别检测溶剂标准和加标尿液样品, 根据待测物各离子对的色谱峰分离和质谱响应情况确定定量、定性离子, 响应值最高的为定量离子, 依次排序作为定性离子。在加标尿样中, MEHHP-<sup>13</sup>C<sub>4</sub> 的定量离子对选择 297.25/145.15 时, 各离子对的相对丰度比与标准溶液中明显不同, 偏差超过 50%, 分析可能存在干扰, 导致无法准确定量。因此 MEHHP-<sup>13</sup>C<sub>4</sub> 的定量、定性离子重新选择响应次低的 124.00 和 79.05。质谱参数见表 2。

**2.2 色谱条件优化** 反相 C<sub>18</sub> 柱是在 mPAEs 分析中

最常用的色谱柱, 尽管 C<sub>8</sub>、C<sub>12</sub>、苯基柱<sup>[14]</sup>、五氟苯基柱<sup>[14,27]</sup> 等也有报道。本实验选择 C<sub>18</sub> 色谱柱作为分离柱。流动相通常选用有机相为甲醇或乙腈<sup>[28]</sup>, 以课题组已有前期研究为基础, 参考既往文献常用的流动相<sup>[29-30]</sup>, 分别比较了 10 ng/ml 混合标准溶液以及尿液样加标溶液在 0. 10% 乙酸 - 乙腈溶液 - 0. 10% 乙酸水溶液、0. 10% 氯化甲醇 - 0. 10% 氨水溶液和乙腈 - 水溶液作为流动相的情况。结果显示, 在中性条件下 mPAEs 的色谱峰会呈现不同程度的展宽或拖尾, 无法获得良好峰形; 在碱性流动相条件下, 色谱峰型和分离均不理想; 在酸性流动相条件下, 不仅峰形良好, 且响应灵敏。最终选择 0. 10% 乙酸乙腈 - 0. 10% 乙酸水溶液为流动相。此外, 还探究了甲酸铵和乙酸铵为缓冲盐对实验结果的影响, 结果显示, 加入缓冲盐后, 各化合物的响应灵敏度下降, 故不添加缓冲盐。

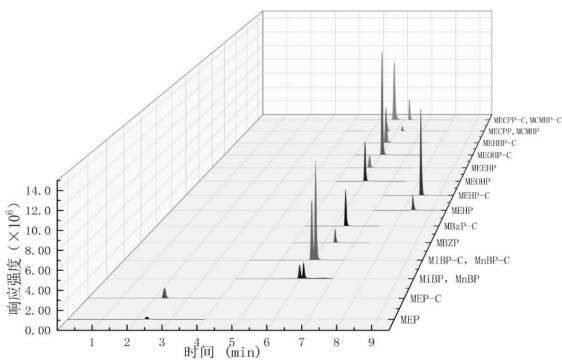


图 1 mPAEs 及其内标的定量离子流图

Fig. 1 The chromatogram of mPAEs and their quantitative ions with isotope - labeled internal standards

**2. 3 液液萃取条件优化** 尿中 mPAEs 常与葡萄糖醛酸等化合物共价结合, 因此, 尿液在萃取净化前是需要酶解的。酶解的条件参照课题组的前期研究<sup>[31]</sup>。对于萃取条件的考察优化, 采用浓度为 10 ng/ml 加标样品的回收率来判断。

**2. 3. 1 萃取试剂种类选择** 9 种待测物的 log P 值范围为 1. 66 ~ 4. 66, 即大部分为中等极性, 故采用了 2 种常用的中等极性有机溶剂乙酸乙酯 (log P = 0. 71) 和二氯甲烷 (log P = 1. 19) 作为萃取剂进行实验。取用多批次尿液样本制备混合尿样, 并加入混合标准溶液制成加标样品。取尿样 1 ml, 分别采用乙酸乙酯、二氯甲烷 3ml 进行液液萃取, 取上清液 40 °C 水浴氮吹蒸发浓缩至近干, 加入 200 μl 50% 乙腈复溶, 取上清液用于色谱质谱分析, 结果如图 2 所示。对于 mPAEs, 在使用乙酸乙酯作为萃取剂时的回收率明显高于二氯甲烷, 且 MEP 在使用二氯甲烷萃取时几乎

不出峰, MECPP, MCMHP 在二氯甲烷中的萃取回收率低于 5%。此外, 用乙酸乙酯萃取时, 待测物被萃取至上层有机相, 而二氯甲烷萃取剂在样液下层, 两相之间悬浮的组织碎屑会加大吸取下层溶液的难度, 结合实验结果以及操作性, 采用乙酸乙酯为萃取剂。

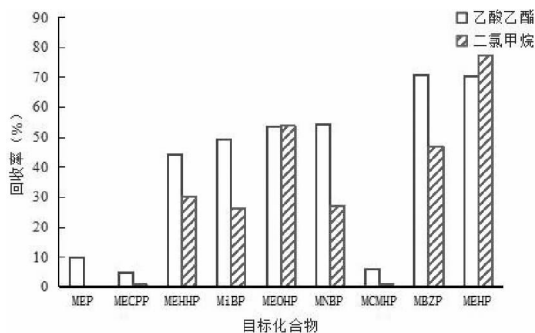


图 2 不同萃取剂的 mPAEs 回收率

Fig. 2 The recovery rates of mPAEs for different extractants

**2. 3. 2 萃取 pH 条件优化** 萃取剂的 pH 值会影响萃取过程中的化学平衡、相互作用和分配系数。不同 pH 条件下, 目标待测物的功能基团可能会发生质子化或去质子化, 从而影响有机溶剂的萃取效率。研究初步选择在加标样品中分别加入甲酸、乙酸铵水溶液 (pH = 6. 0)、氨水, 使样液呈酸、中、碱性后进行萃取和测定, 观察萃取 pH 是否对分析有影响。与萃取试剂优化实验一样, 取用已经提前制备好的多批次尿液和加标样, 分别加入 60 μl 甲酸、60 μl 乙酸铵水、60 μl 0. 25% 氨水, 涡旋混匀后加入乙酸乙酯 3 ml 对样品进行液液萃取, 浓缩复溶后, 取上清液进行色谱质谱分析, 结果如图 3 所示。对于 mPAEs, 酸性条件下的响应和峰面积均远远高于中性和碱性, 分析原因是 mPAEs 为酸性物质, 在碱性条件下更多以离子状态存在, 容易带上电荷, 具有更高的极性和亲水性, 不易被乙酸乙酯萃取。因此用甲酸酸化尿样, 并进一步对甲酸加入量进行优化。

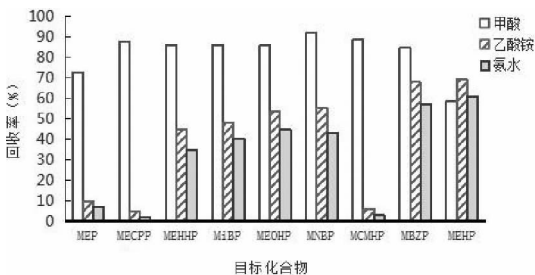


图 3 样液酸碱对 mPAEs 回收率的影响

Fig. 3 Effect of sample solution pH on the recovery of mPAEs

在乙酸乙酯萃取前分别用不同浓度的甲酸 - 水调节样液 pH 值,使甲酸浓度为 0%、0.01%、2.00%、20% (v/v),按照甲酸浓度和加入量估算 pH 值,分别为 pH ≈ 1、pH ≈ 2、pH ≈ 4、pH ≈ 6。图 4 可见,对于 mPAEs,加入甲酸可提高加标回收率,酸性对除 MEHP 外的 mPAEs 萃取效率提高更明显,pH ≈ 2 时所有 mPAEs 的峰面积均大于其他 pH。最终选择 2% 甲酸 (pH ≈ 2) 作为萃取介质酸度。

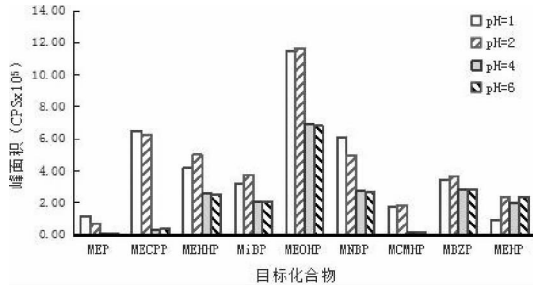


图 4 样液 pH 对 mPAEs 峰面积的影响

Fig. 4 Effect of sample solution pH on the peak area of mPAEs

**2.3.3 萃取剂用量条件优化** 试验了乙酸乙酯不同用量 (2 ~ 5 ml) 对萃取效率的影响,结果如图 5。3 ml 与 4 ml 的萃取效率均高,综合考虑,选择 3 ml 乙酸乙酯用于尿液样品的萃取。

200 ng/ml 浓度范围内线性良好,相关系数均大于 0.999。方法 LODs 和 LOQs 的判定分别为 3 倍信噪比和 10 倍信噪比,结果见表 3。取样液 1 ml 进行计算,PAEs 类化合物的 LODs 和 LOQs 分别为  $1.5 \times 10^{-3} \sim 0.29$  ng/ml 和  $4.9 \times 10^{-3} \sim 0.98$  ng/ml。

**2.4.2 准确度和精密度** 取若干尿样混合后,分别加入低、中、高 (2.00、4.00、20.00 ng/ml, MBzP 为 0.20、2.00、4.00 ng/ml) 三个水平的混合标准溶液,内标浓度均为 4.00 ng/ml。每日重复 3 次,连续 3 天进行检测,计算所得平均回收率,并计算日内精密度和日间精密度,结果见表 3。实验所测的 9 种 mPAEs 都有对应的同位素内标,可校正实验中产生的误差及基质效应带来的影响。尿中 9 种 mPAEs 的三个水平回收率为 87.05% ~ 111.50%,日内精密度为 0.31% ~ 3.05%,日间精密度为 0.59% ~ 3.46%。

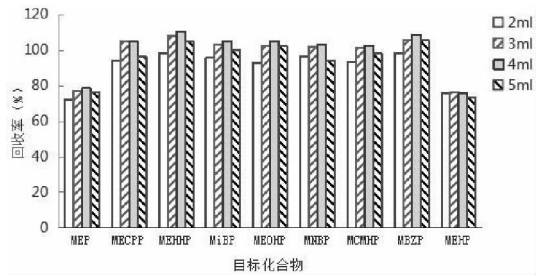


图 5 萃取剂用量对 mPAEs 回收率的影响

Fig. 5 Effect of extractant volume on the recovery rates of mPAEs

**2.4 方法学指标评估**

**2.4.1 线性范围、检出限和定量限** PAEs 在 0.50 ~

表 3 方法性能指标 (n = 3)

Table 3 The performance parameters of the method (n = 3)

化合物	本底值 (ng/ml)	加标水平 (ng/ml)	回收率 (%)	RSD (%)		回归方程	相关系数 (r)	LOD (ng/ml)	LOQ (ng/ml)
				日内	日间				
MEP	8.25	2.00	105.21 ± 0.33	0.31	1.41	$y = 5.79 \times 10^{-2}x + 2.70 \times 10^{-3}$	0.999 7	$0.28 \times 10^{-2}$	$0.95 \times 10^{-1}$
		4.00	111.53 ± 1.22	1.09	0.96				
		20.00	109.12 ± 0.99	0.91	1.45				
MECPP	5.32	2.00	100.21 ± 0.51	0.51	1.64	$y = 5.21 \times 10^{-2}x + 1.60 \times 10^{-1}$	0.999 7	$0.38 \times 10^{-1}$	0.13
		4.00	103.94 ± 1.04	1.00	0.68				
		20.00	105.72 ± 0.75	0.71	0.59				
MEHHP	5.90	2.00	97.23 ± 2.45	2.52	1.83	$y = 5.76 \times 10^{-2}x + 8.74 \times 10^{-2}$	0.999 9	$0.93 \times 10^{-2}$	$0.31 \times 10^{-1}$
		4.00	103.87 ± 2.02	1.94	3.46				
		20.00	107.61 ± 3.10	2.88	2.82				
MiBP	3.65	2.00	91.35 ± 1.37	1.50	1.53	$y = 1.30 \times 10^{-1}x - 6.40 \times 10^{-3}$	0.999 0	$0.14 \times 10^{-1}$	$0.47 \times 10^{-1}$
		4.00	96.78 ± 1.48	1.53	1.06				
		20.00	99.89 ± 1.14	1.14	1.87				
MEOHP	8.96	2.00	95.98 ± 1.68	1.75	2.28	$y = 3.61 \times 10^{-2}x + 2.52 \times 10^{-1}$	0.999 4	$0.18 \times 10^{-2}$	$0.60 \times 10^{-2}$
		4.00	98.18 ± 1.71	1.74	2.17				
		20.00	102.42 ± 1.64	1.60	2.18				
MNBP	2.53	2.00	87.05 ± 0.54	0.62	1.71	$y = 8.73 \times 10^{-2}x + 3.00 \times 10^{-3}$	0.999 8	$0.26 \times 10^{-1}$	$0.86 \times 10^{-1}$
		4.00	110.91 ± 0.89	0.80	1.61				
		20.00	111.41 ± 2.18	1.96	1.52				
MCMHP	45.20	2.00	96.24 ± 0.64	0.66	2.33	$y = 9.20 \times 10^{-2}x - 8.40 \times 10^{-3}$	0.999 8	0.29	0.98
		4.00	100.52 ± 1.72	1.71	1.33				
		20.00	106.74 ± 0.41	0.38	2.94				

(续表)

化合物	本底值 (ng/ml)	加标水平 (ng/ml)	回收率 (%)	RSD(%)		回归方程	相关系数 (r)	LOD (ng/ml)	LOQ (ng/ml)
				日内	日间				
MBZP	0.16	0.20	96.38 ± 2.94	3.05	1.61	$y = 6.43 \times 10^{-2}x + 4.00 \times 10^{-4}$	0.999 7	$0.24 \times 10^{-2}$	$0.79 \times 10^{-2}$
		2.00	97.12 ± 1.78	1.83	2.92				
		4.00	99.03 ± 1.81	1.83	1.76				
MEHP	4.52	2.00	99.82 ± 0.79	0.79	1.53	$y = 3.92 \times 10^{-2}x - 9.40 \times 10^{-3}$	0.999 8	$0.15 \times 10^{-2}$	$0.49 \times 10^{-2}$
		4.00	105.51 ± 0.50	0.47	2.33				
		20.00	107.41 ± 1.08	1.01	0.89				

**2.5 尿中 mPAEs 的检测** 对来自华西医院健康体检的 277 个正常人尿样进行了检测,结果见表 4,由于数据均为偏态,因此用中位数及上下四分位数进行统计描述。9 种 mPAEs 中除 MBzP 以外,其余 8 种 mPAEs 的检出率均为 100%。MBzP 的检出率为

87.73%,检出水平为 0.037 μg/g<sub>肌酐</sub>(中位数)。结果显示,PAEs 的人群暴露是广泛的,暴露水平不低,尤其是 MnBP,暴露水平远高于其他国家和地区<sup>[32-36]</sup>,提示其风险监测和评估的必要性和紧迫性。

表 4 尿样中 mPAEs 的检出结果(μg/g<sub>肌酐</sub>)

Table 4 Detection results of mPAEs metabolites in urine samples (μg/g creatinine)

化合物	检出范围值	检出率(%)	第 25 百分位数	中位数	第 75 百分位数
MEP	0.56 ~ 1.38 × 10 <sup>3</sup>	100.00	5.69	9.80	19.87
MEHP	0.12 ~ 55.25	100.00	2.30	3.34	5.88
MEHHP	2.37 ~ 85.92	100.00	5.14	7.48	10.97
MEOHP	0.62 ~ 24.92	100.00	1.72	2.53	3.70
MECPP	3.21 ~ 83.15	100.00	8.89	12.91	18.20
MCMHP	0.43 ~ 24.61	100.00	2.34	3.57	5.06
MnBP	19.17 ~ 1.66 × 10 <sup>3</sup>	100.00	125.30	194.10	294.30
MiBP	2.87 ~ 199.63	100.00	13.49	21.46	31.71
MBzP	ND ~ 3.40	87.73	0.03	0.04	0.08

注:ND,not detected,未检出。

### 3 结论

本研究建立了尿液中 9 种 mPAEs 的液液萃取 - 超高效液相色谱 - 串联质谱法,并成功应用于 277 个正常人尿样中的 mPAEs 的检测,本方法可为人群 PAEs 暴露水平及相关研究提供技术支持。

**利益冲突声明** 本研究不存在任何利益冲突

### 参考文献

[1] Ahmadi E, Yousefzadeh S, Ansari M, et al. Performance, kinetic, and biodegradation pathway evaluation of anaerobic fixed film fixed bed reactor in removing phthalic acid esters from wastewater[J]. Scientific Reports, 2017, 7: 41020.

[2] Heudorf U, Mersch - Sundermann V, Angerer J. Phthalates: toxicology and exposure[J]. International Journal of Hygiene and Environmental Health, 2007, 210(5): 623 - 634.

[3] Bui TT, Giovanoulis G, Cousins AP, et al. Human exposure, hazard and risk of alternative plasticizers to phthalate esters[J]. Science of the Total Environment, 2016, 541: 451 - 467.

[4] Toft G, Jönsson BAG, Lindh CH, et al. Association between pregnancy loss and urinary phthalate levels around the time of conception[J]. Environmental Health Perspectives, 2012, 120(3): 458 - 463.

[5] 邱爽,宋明明,刘畅.邻苯二甲酸酯的生殖毒性及分子机制研究进展[J].生命科学,2023,35(7):935 - 946.  
Qiu S, Song MM, Liu C. Research progress on reproductive toxicity and molecular mechanism of phthalates esters[J]. Chinese Bulletin of Life Sciences, 2023, 35(7): 935 - 946. (In Chinese)

[6] Zhang YJ, Guo JL, Xue JC, et al. Phthalate metabolites: Characterization, toxicities, global distribution, and exposure assessment[J]. Environmental Pollution, 2021, 291: 118106.

[7] Dueñas - Moreno J, Mora A, Cervantes - Avilés P, et al. Groundwater contamination pathways of phthalates and bisphenol A: origin, characteristics, transport, and fate - A review[J]. Environment International, 2022, 170: 107550.

[8] Xiong YH, Pei DS. A review on efficient removal of phthalic acid esters via biochars and transition metals - activated persulfate systems[J]. Chemosphere. 2021, 277: 130256.

[9] Chen M, Tao L, Collins EM, et al. Simultaneous determination of multiple phthalate metabolites and bisphenol - A in human urine by liquid chromatography - tandem mass spectrometry[J]. Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences, 2012, 904: 73 - 80.

[10] 王亚,肖霞霞,杨云,等.溶剂萃取 - 气相色谱 - 三重四极杆串联质谱法测定水体和土壤中 6 种邻苯二甲酸酯[J].分析科学学报,2023,39(3):287 - 294.  
Wang Y, Xiao XX, Yang Y, et al. Determination of six phthalic acid esters in water and Soil samples by gas Chromatography -

- Tandem mass spectrometry with solvent extraction method [J]. *Journal of Analytical Science*, 2023, 39(3): 287 - 294. (In Chinese)
- [11] 吴爱英, 宋纯艳, 张辰辰, 等. 气相色谱 - 串联质谱法同时测定白酒中 17 种邻苯二甲酸酯类塑化剂含量[J]. *中国酿造*, 2023, 42(4): 250 - 255.
- Wu AY, Song CY, Zhang CC, et al. Simultaneous determination of 17 kinds of phthalate plasticizers in Baijiu based on GC - MS/MS [J]. *China Brewing*, 2023, 42(4): 250 - 255. (In Chinese)
- [12] 张迪, 岳青阳, 李尚颖, 等. GC - MS 法测定吡拉西坦氯化钠注射液 21 种邻苯二甲酸酯类塑化剂[J]. *中南药学*, 2022, 20(9): 2147 - 2150.
- Zhang D, Yue QY, Li SY, et al. Determination of 21 phthalic acid ester plasticizers in piracetam Sodium chloride injection by GC - MS [J]. *Central South Pharmacy*, 2022, 20(9): 2147 - 2150. (In Chinese)
- [13] 王小艳, 张涛. 气相色谱 - 质谱法检测中药液袋中 16 种邻苯二甲酸酯[J]. *中国药业*, 2022, 31(9): 98 - 101.
- Wang XY, Zhang T. Determination of 16 kinds of phthalates in traditional Chinese medicine liquid Bags by GC - MS [J]. *China Pharmaceuticals*, 2022, 31(9): 98 - 101. (In Chinese)
- [14] Silva MJ, Samandar E, Preau JL, et al. Quantification of 22 phthalate metabolites in human urine [J]. *Journal of Chromatography B*, 2007, 860(1): 106 - 112.
- [15] 王东旭, 王新财, 胡奇杰, 等. 盐析辅助液液萃取/超高效液相色谱 - 串联质谱法测定含乳饮料中 12 种邻苯二甲酸酯[J]. *分析测试学报*, 2022, 41(10): 1516 - 1522.
- Wang DX, Wang XC, Hu QJ, et al. Determination of 12 phthalate esters in milk beverage using ultra performance liquid chromatography - Tandem mass spectrometry with salting - out assisted Liquid - Liquid extraction [J]. *Journal of Instrumental Analysis*, 2022, 41(10): 1516 - 1522. (In Chinese)
- [16] Frigerio G, Campo L, Mercadante R, et al. Development and validation of a liquid chromatography/tandem mass spectrometry method to quantify metabolites of phthalates, including di - 2 - ethylhexyl terephthalate (DEHTP) and bisphenol A, in human urine [J]. *Rapid Communications in Mass Spectrometry*, 2020, 34(13): e8796.
- [17] 薄艳娜, 李蓉, 张朋杰, 等. 高效液相色谱 - 串联质谱法测定焙烤食品及其塑料包装中 31 种邻苯二甲酸酯[J]. *色谱*, 2016, 34(9): 868 - 879.
- Bo YN, Li R, Zhang PJ, et al. Determination of 31 phthalate esters in baked foods and plastic packaging materials by high performance chromatography - tandem mass spectrometry [J]. *Chinese Journal of Chromatography*, 2016, 34(9): 868 - 879. (In Chinese)
- [18] 吴敏, 郑向华, 张志刚, 等. 前吸附技术 - 超高压液相色谱 - 串联质谱法同时测定酒中 24 种邻苯二甲酸酯[J]. *色谱*, 2016, 34(7): 673 - 680.
- Wu M, Zheng XH, Zhang ZG, et al. Simultaneous determination of 24 phthalates in liquors using front adsorption technology and ultra performance liquid chromatography - tandem mass spectrometry [J]. *Chinese Journal of Chromatography*, 2016, 34(7): 673 - 680. (In Chinese)
- [19] 徐海娥, 徐吉巧, 戴雪璐, 等. 固相萃取 - 气相色谱 - 质谱法测定血清邻苯二甲酸酯类化合物含量[J]. *实验室检测*, 2024, 2(11): 29 - 32.
- Xu HE, Xu JQ, Dai XL, et al. Determination of phthalate esters in serum by solid - phase extraction - gas chromatography - mass spectrometry [J]. *Laboratory Testing*, 2024, 2(11): 29 - 32. (In Chinese)
- [20] Shi YP, Zhang L, Shao J, et al. Facile preparation of activated Carbon from peanut shell for determination of bisphenol a in human urine by High - Performance liquid chromatography [J]. *Journal of Nanoscience and Nanotechnology*, 2021, 21(3): 1439 - 1445.
- [21] Salazar - Beltrán D, Hinojosa - Reyes L, Ruiz - Ruiz E, et al. Determination of phthalates in bottled water by automated on - line solid phase extraction coupled to liquid chromatography with UV detection [J]. *Talanta*, 2017, 168: 291 - 297.
- [22] 管悦, 泮燕妮. 液液萃取气相色谱 - 串联质谱法测定含油半固体食品中 19 种邻苯二甲酸酯类化合物[J]. *化学分析计量*, 2024, 33(4): 90 - 94, 100.
- Guan Y, Pan YM. Determination of 19 phthalate esters in oily semi - solid foods by liquid - liquid extraction combined with GC - MS/MS [J]. *Chemical Analysis and Meterage*, 2024, 33(4): 90 - 94, 100. (In Chinese)
- [23] 林家宝, 孙雨豪, 王建, 等. 土壤中邻苯二甲酸酯的高效液相色谱检测方法[J]. *中国环境科学*, 2023, 43(2): 756 - 763.
- Lin JB, Sun YH, Wang J, et al. Study on the high - performance liquid chromatography detection method of phthalic acid esters in soils [J]. *China Environmental Science*, 2023, 43(2): 756 - 763. (In Chinese)
- [24] 刘喜珍. 环境水体中邻苯二甲酸二(丁、2 - 乙基己基)酯的高效液相色谱分析方法研究[J]. *绿色科技*, 2022, 24(12): 84 - 87.
- Liu XZ. Study on analytically methods of dibutyl phthalate and Di (2 - ethylhexyl ) phthalate in environmental water by high performance liquid chromatography [J]. *Journal of Green Science and Technology*, 2022, 24(12): 84 - 87. (In Chinese)
- [25] 孙倩, 戴浩强, 陈佩佩, 等. 多功能离子液体分散液液微萃取结合高效液相色谱法检测人尿中 5 种邻苯二甲酸酯代谢物[J]. *色谱*, 2020, 38(8): 929 - 936.
- Sun Q, Dai HQ, Chen PP, et al. Combination of dispersive liquid - liquid microextraction using multifunctional Ionic liquids with high performance chromatography for determination of phthalate ester metabolites in human urine sample [J]. *Chinese Journal of Chromatography*, 2020, 38(8): 929 - 936. (In Chinese)
- [26] ICH Harmonisation for Better Health. ICH M10 guideline: Bioanalytical method validation and study sample analysis [EB/OL]. [2025 - 05 - 11]. <https://www.pmda.go.jp/files/000249612.pdf>.
- [27] Miao HJ, Huang Y, Ma C, et al. Ultra - High - Performance liquid Chromatography - Isotope dilution tandem mass spectrometry for the determination of phthalate secondary metabolites in human serum based on Solid - Phase extraction [J]. *Journal of AOAC International*, 2018: 1178 - 1185.
- [28] Philips EM, Jaddoe VWV, Asimakopoulos AG, et al. Bisphenol and phthalate concentrations and its determinants among pregnant women in a population - based cohort in the Netherlands, 2004 - 5 [J]. *Environmental Research*, 2018, 161: 562 - 572.

- [8] Feng L, Nyunt MSZ, Feng L, et al. Frailty predicts new and persistent depressive symptoms among community – dwelling older adults: findings from Singapore longitudinal aging study [J]. *Journal of the American Medical Directors Association*, 2014, 15 (1): 76. e7 – 76. e12.
- [9] Salaffi F, De angelis R, Farah S, et al. Frailty as a novel predictor of achieving comprehensive disease control (CDC) in rheumatoid arthritis [J]. *Clinical Rheumatology*, 2021, 40 (12): 4869 – 4877.
- [10] 陈蕾. 社会经济地位对中国老年人虚弱指数和虚弱轨迹的影响 – 基于生命历程的视角 [J]. *中国社会科学院大学学报*, 2023, 43(4): 83 – 102.  
Chen L. The impact of socioeconomic status on the frailty index and frailty trajectories of Chinese older adults: A life course – based perspective [J]. *Journal of Chinese Academy of Social Sciences University*, 2023, 43(4): 83 – 102. (In Chinese)
- [11] Dai HJ, Li F, Bragazzi NL, et al. Distinct developmental trajectories of body mass index and diabetes risk: A 5 – year longitudinal study of Chinese adults [J]. *Journal of Diabetes Investigation*, 2020, 11(2): 466 – 474.
- [12] Aggio D, Papachristou E, Papacosta O, et al. Trajectories of physical activity from midlife to old age and associations with subsequent cardiovascular disease and all – cause mortality [J]. *Journal of Epidemiology and Community Health*, 2020, 74(2): 130 – 136.
- [13] Peristera P, Platts LG, Magnusson hanson LL, et al. A comparison of the B – spline group – based trajectory model with the polynomial group – based trajectory model for identifying trajectories of depressive symptoms around old – age retirement [J]. *Aging & Mental Health*, 2020, 24(3): 445 – 452.
- [14] Chamberlain AM, St sauver JL, Jacobson DJ, et al. Social and behavioural factors associated with frailty trajectories in a population – based cohort of older adults [J]. *BMJ Open*, 2016, 6(5): e011410.
- [15] Germonpré S, Mulier S, Falzon L, et al. Prevalence of frailty and pre – frailty in patients with rheumatoid arthritis: a systematic literature review and meta – analysis [J]. *Clinical and Experimental Rheumatology*, 2023, 41(7): 1443 – 1450.
- [16] Dent E, Morley JE, Cruz – Jentoft AJ, et al. Physical frailty: ICF SR international clinical practice guidelines for identification and management [J]. *Journal of Nutrition Health & Aging*, 2019, 23(9): 771 – 787.
- [17] Welstead M, Luciano M, Russ TC, et al. Heterogeneity of frailty trajectories and associated factors in the Lothian birth cohort 1936 [J]. *Gerontology*, 2022, 68(8): 861 – 868.
- [18] Chu WM, Ho HE, Yeh CJ, et al. Self – rated health trajectory and frailty among community – dwelling older adults: evidence from the Taiwan Longitudinal Study on Aging (TL SA) [J]. *BMJ Open*, 2021, 11(8): e049795.
- [19] Soysal P, Stubbs B, Lucato P, et al. Inflammation and frailty in the elderly: A systematic review and meta – analysis [J]. *Ageing Research Reviews*, 2016, 31: 1 – 8.
- [20] Franceschi C, Campisi J. Chronic Inflammation (inflammaging) and its potential contribution to age – associated diseases [J]. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, 2014, 69 Suppl 1: S4 – S9.
- [21] Kapse B, Budev MM, Singer JP, et al. Immune aging: biological mechanisms, clinical symptoms, and management in lung transplant recipients [J]. *Frontiers in Transplant*, 2024, 3: 1356948.
- [22] Xia S, Zhang X, Zheng S, et al. An update on inflamm – aging: Mechanisms, prevention, and treatment [J]. *Journal of Immunology Research*, 2016, 2016: 8426874.
- [23] El assar M, Angulo J, Rodríguez – Mañas L. Frailty as a phenotypic manifestation of underlying oxidative stress [J]. *Free Radical Biology and Medicine*, 2020, 149: 72 – 77.
- [24] Baker JF, Mostoufi – Moab S, Long J, et al. Intramuscular fat accumulation and associations with body composition, strength, and physical functioning in patients with rheumatoid arthritis [J]. *Arthritis Care & Research*, 2018, 70(12): 1727 – 1734.
- [25] Spies CM, Straub RH, Cutolo M, et al. Circadian rhythms in rheumatology – a glucocorticoid perspective [J]. *Arthritis Research & Therapy*, 2014, 16(2): S3.

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- [29] Vela – Soria F, Ballesteros O, Zafra – Gómez A, et al. A multiclass method for the analysis of endocrine disrupting chemicals in human urine samples. Sample treatment by dispersive liquid – liquid microextraction [J]. *Talanta*, 2014, 129: 209 – 218.
- [30] Guo T, Meng XY, Liu XK, et al. Associations of phthalates with prostate cancer among the US population [J]. *Reproductive Toxicology*, 2023, 116: 108337.
- [31] Wang X, Hu ZF, Jin YM, et al. Exploring the relationships between exposure levels of bisphenols and phthalates and prostate cancer occurrence [J]. *Journal of Hazardous Materials*, 2024, 474: 134736.
- [32] Minister of Health. Sixth report on human biomonitoring of environmental chemicals in Canada [EB/OL]. [2025 – 05 – 11]. <https://www.canada.ca/content/dam/hc-sc/documents/services/environmental-workplace-health/reports-publications/environmental-contaminants/sixth-report-human-biomonitoring/pub1-eng.pdf>.
- [33] Dualde P, León N, Sanchis Y, et al. Biomonitoring of phthalates, bisphenols and parabens in children: exposure, predictors and risk assessment [J]. *International Journal of Environmental Research and Public Health*, 2021, 18(17): 8909.
- [34] Centers for Disease Control and Prevention of U. S. . National report on human exposure to environmental chemicals [EB/OL]. [2025 – 05 – 11]. <https://www.cdc.gov/biomonitoring/resources/national-exposure-report.html>
- [35] Jacobson MH, Hamra GB, Monk C, et al. Prenatal exposure to nonpersistent environmental chemicals and postpartum depression [J]. *JAMA Psychiatry*, 2024, 81(1): 67 – 76.
- [36] Al – Saleh I, Elkhatib R, Alnuwaysir H, et al. The cumulative risk assessment of phthalates exposure in preterm neonates [J]. *International Journal of Hygiene and Environmental Health*, 2023, 248: 114112.

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