

Different Analytical Techniques, Pretreatment Methods and Adsorbent Materials for the Determination of Trans, Trans-Muconic Acid in Urine Samples

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Abstract: Benzene is a human carcinogen and its metabolite, Trans, trans-muconic acid (tt-MA) has been introduced by some environmental and occupational health regulatory associations as a biological index for the assessment of benzene exposure. This paper summarized and compared different analytical techniques, pretreatment methods and adsorbent materials for the determination of tt-MA in urine samples.

1. Introduction

Benzene is a major industrial chemical that has been used in numerous production and synthesis processes in the petrochemical, paint, plastic, detergents, drugs, rubber and dye industries [1-3]. According to the International Agency for Research on Cancer (IARC), benzene is classified as a group I human carcinogen [4, 5]. Long-term occupational and environmental exposure to benzene may cause bone marrow damage, leukemia, aplastic anemia and so on [6]. Trans, trans-muconic acid (tt-MA) and S-phenylmercapturic acid (S-PMA) are the most commonly used urinary biomarkers associated with benzene exposure in humans to be detected from urine or blood [7-9]. The American Conference of Governmental Industrial Hygienists (ACGIH) has recommended tt-MA as a biological exposure index (BEI) which is suitable urinary biomarker of benzene at high concentrations (above 1 ppm) [1, 10-12]. It appears that tt-MA is a more specific biomarker than other metabolites at high levels of benzene exposure.

Several analytical methods, including high-performance liquid chromatography (HPLC), gas chromatography (GC), ultraviolet and visible spectrophotometry (UV-vis) and mass spectrometry (MS) have been applied for the determination of tt-MA in urine [6, 12-17]. However, owing to the low levels of tt-MA in urine samples and the interferences in complex matrixes, extraction and preconcentration processes prior to detection is necessary [18, 19]. Solid-phase extraction (SPE) and liquid-liquid extraction (LLE) are the most widely-used sample pretreatment methods for extracting and enriching tt-MA from urine samples [20]. It is obvious the choice of appropriate adsorbent materials is a key factor for effective extraction of tt-MA. Recently, there are several types of adsorbents for SPE including metal-organic framework [12], molecularly imprinted polymers [21] and other special adsorbents.

This paper summarized and compared different analytical techniques, pretreatment methods and adsorbent materials for the determination of tt-MA in urine samples.

2. Different analytical techniques for the determination of tt-MA in urine samples

Various analytical approaches have been introduced to measure tt-MA in urine samples, including high-performance liquid chromatography (HPLC), gas chromatography (GC), ultraviolet and visible spectrophotometry (UV-vis) and mass spectrometry (MS). Bhandari et al. [7] developed an ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method for simultaneously measuring 8 biomarkers of volatile organic compound (VOC) exposure, including

tt-MA. Ruppert et al. [14] developed a sensitive and specific method for the determination of tt-MA in urine analyzed by gas chromatography-mass spectrometry (GC-MS). Marchese et al. [15] demonstrated the use of a liquid chromatography/hybrid quadrupole time-of-flight (QqTOF) mass spectrometry instrument for simultaneous determination of urinary metabolites of benzene, toluene, xylene and styrene (the metabolites selected including tt-MA). Tranfo et al. [16] presented a HPLC/MS/MS method for reliable quantitative determination of t,t-MA. Olmos et al. [22] described a specific and precise high-performance liquid chromatography (HPLC) method for the quantification of tt-MA in human urine. Lee et al. [23] described a HPLC-DAD method for tt-MA determination with enhanced selectivity and sensitivity. As shown in Table 1, tt-MA was usually determined by HPLC methods with detection by either UV or, more recently, electrospray tandem mass spectrometry and the detection limits were found to be very low.

Table 1. Different analytical techniques for the determination of tt-MA in urine samples.

| Analyte | Linearity ranges | Techniques | LODs | References |
|---------|------------------|----------------------|------------|------------|
| tt-MA | 0.759-759 ng/mL | UPLC-MS/MS | 1.20 ng/mL | [7] |
| | 0-200µg/mL | Luminescence spectra | 0.1µg/mL | [12] |
| | 0.01-1.00 µg/mL | GC-MS | 0.01 µg/mL | [14] |
| | 5-1000 ng/mL | HPLC-MS/MS | 1.0 ng/mL | [15] |
| | 20-1000 ng/mL | HPLC-MS/MS | 0.22 ng/mL | [16] |
| | 10-2000 ng/mL | HPLC-UV | 10.8 ng/mL | [22] |
| | 5-5000 ng/mL | HPLC-DAD | 5.0 ng/mL | [23] |

3. Different pretreatment methods for the determination of tt-MA in urine samples

The direct analysis of tt-MA in urine is not recommended owing to the low levels of tt-MA and the complexity of biological matrixes. Therefore, extraction and preconcentration steps in the analytical procedure is necessary to enhance the sensitivity and selectivity. Bahrami et al. [19] developed a novel technique for extraction and determination of trans,trans-muconic acid (tt-MA), hippuric acid (HA) and mandelic acid (MA) by means of ion-pair-based hollow fiber liquid-phase microextraction (IP-HF-LPME). Mudiam et al. [24] reported a MIP using methacrylic acid (MAA) as a monomer followed by simultaneous derivatization using ethyl chloroformate and pre-concentration using dispersive liquid-liquid microextraction (DLLME) for the determination of tt-MA in urine samples. Gomes et al. [25] developed an analytical method for the determination of urinary tt-MA using solid phase extraction (SPE) onto a Strata SAX cartridge. Rismanchian et al. [26] detected tt-MA in urine samples using partitioned dispersive liquid-liquid microextraction technique (PDLLME) coupled with high-performance liquid chromatography-UV detection. Chauhan et al. [27] synthesized a molecularly imprinted polymer (MIP) and used it as an adsorbent for solid phase extraction of six urinary benzene metabolites (UBMs). Table 2 clearly demonstrates that SPE and LLE are the most widely-used sample pretreatment methods in the analysis of tt-MA in urine samples.

Table 2. Different pretreatment methods for the determination of tt-MA in urine samples.

| Analyte | Sample pretreatment methods | Techniques | LODs | References |
|---------|-----------------------------|------------|-------------|------------|
| tt-MA | IP-HF-LPME | HPLC-UV | 0.0001µg/mL | [19] |
| | SPE | HPLC-UV | 5.0 µg/L | [23] |
| | MIP-DLLME | GC-MS | 0.037 µg/mL | [24] |
| | SPE | HPLC-UV | 0.11 ng/mL | [25] |
| | PDLLME | HPLC-UV | 0.11 ng/mL | [26] |
| | MISPE | GC-MS | 1.2 ng/mL | [27] |

4. Different adsorbent materials for the determination of tt-MA in urine samples

Due to the key role of the adsorbents in SPE technique, new types of SPE adsorbents to improve the extraction efficiency for tt-MA were explored. Qu et al. [12] synthesized a luminescent lanthanide-based MOFs hybrid material (Tb(III)@MOF-SO₃) as a fluorescence sensor based on fluorescence quenching mechanisms to detect tt-MA in a urine system. Soleimani et al. [21] developed a novel approach based on a molecularly imprinted polymer (MIP) in microextraction by packed sorbent (MEPS), followed by high-performance liquid chromatography with ultra-violet detection (MIMEPS-HPLC-UV) to determine tt-MA in urine samples. Vieira et al. [28] synthesized a MIP (using 4-vinylpyridine as functional monomer) based on molecularly imprinted solid-phase extraction (MISPE) for the extraction of tt-MA in urine samples, followed by high-performance liquid chromatography with ultraviolet detection (HPLC-UV). Mudiam et al. [24] synthesized a MIP (using methacrylic acid as functional monomer) followed by simultaneous ethyl chloroformate derivatization and preconcentration by dispersive liquid-liquid microextraction (DLLME) for the analysis of tt-MA in urine samples using gas chromatography-mass spectrometry (GC-MS), as shown in Table 3.

Table 3. Different adsorbent materials for the determination of tt-MA in urine samples.

| Analyte | Adsorbent materials | Sample preparation techniques | Techniques | LODs | References |
|---------|-----------------------------|-------------------------------|----------------------|-------------|------------|
| tt-MA | Tb(III)@MOF-SO ₃ | / | Luminescence spectra | 0.1 µg/mL | [12] |
| | MIP | MIMEPS | HPLC-UV | 0.015µg/mL | [21] |
| | MIP | MISPE | HPLC-UV | 0.3 µg/mL | [28] |
| | MIP | DLLME | GC-MS | 0.037 µg/mL | [24] |

5. Conclusion

Many methods have been applied to determine tt-MA in urine samples, including HPLC-UV, GC-MS, luminescence spectra and ect. Among them, HPLC-UV is the most widely-used technique. Due to the complexity of real urine sample matrix, SPE and LLE are frequently applied as sample pretreatment methods. And new types of SPE adsorbent materials are developed to improve the extraction efficiency for tt-MA determination.

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