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Sol–gel-based solid-phase microextraction and gas chromatography-mass spectrometry determination of dextromethorphan and dextrorphan in human plasma

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Abstract

A novel solid-phase microextraction (SPME) method was developed for isolation of dextromethorphan (DM) and its main metabolite dextrorphan (DP) from human plasma followed by GC-MS determination. Three different polymers, poly(dimethylsiloxane) (PDMS), poly(ethylenepropyleneglycol) monobutyl ether (Ucon) and polyethylene glycol (PEG) were synthesized as coated fibers using sol–gel methodologies. DP was converted to its acetyl-derivative prior to extraction and subsequent determination. The porosity of coated fibers was examined by SEM technique. Effects of different parameters such as fiber coating type, extraction mode, agitation method, sample volume, extraction time, and desorption condition, were investigated and optimized. The method is rapid, simple, easy and inexpensive and offers high sensitivity and reproducibility. The limits of detection are 0.010 and 0.015 ng/ml for DM and DP, respectively. The precisions for both analytes are below 5% ($n = 5$). The correlation coefficient was satisfactory ($r^2 > 0.99$) for both DM and DP. Linear ranges were obtained from 0.03 ng/ml to 2 μg/ml for DM and from 0.05 ng/ml to 2 μg/ml for DP.

Keywords: Sol–gel technology; Solid-phase microextraction; Gas chromatography-mass spectrometry; Dextromethorphan; Dextrophan; Plasma samples

1. Introduction

Dextromethorphan (DM) is a safe perorally administered antitussive that is widely available without prescription for which the metabolite fate in humans has been well-characterized (Fig. 1). The metabolism of DM is primarily by O-demethylation to dextrorphan (DP), a reaction that is mediated by cytochrome P450 enzyme CYP2D6. DM is also metabolized to 3-methoxymorphinan and 3-hydroxymorphinan, but these appear to be minor pathways mediated by CYP2D6 and CYP3A3/4, respectively. Because the CYP2D6 enzyme displays polymorphism, DM metabolism to DP has been used to phenotype subjects [1,2].

Several methods have been reported for the determination of DM and DP in biological fluids including high-performance liquid chromatography (HPLC) with UV [3,4], fluorescence detection [5–8,9], and tandem mass spectrometry (MS/MS) [2,10–12], gas chromatography (GC) [1,13,14], capillary electrophoresis [15,16] and thin-layer chromatography (TLC) [17]. However, all of the reported methods, except LC/MS/MS methods suffer from lack of sensitivity and poor limit of quantification. In most of methods described, liquid–liquid extraction (LLE) and solid-phase extraction (SPE) are the most commonly used techniques for isolation and/or enrichment of DM and DP prior to chromatographic analysis. These approaches have disadvantages, as they are tedious, labor-intensive and time-consuming procedures. Although, the use of robotics has greatly decreased the level of tediousness, labor and time required for SPE and

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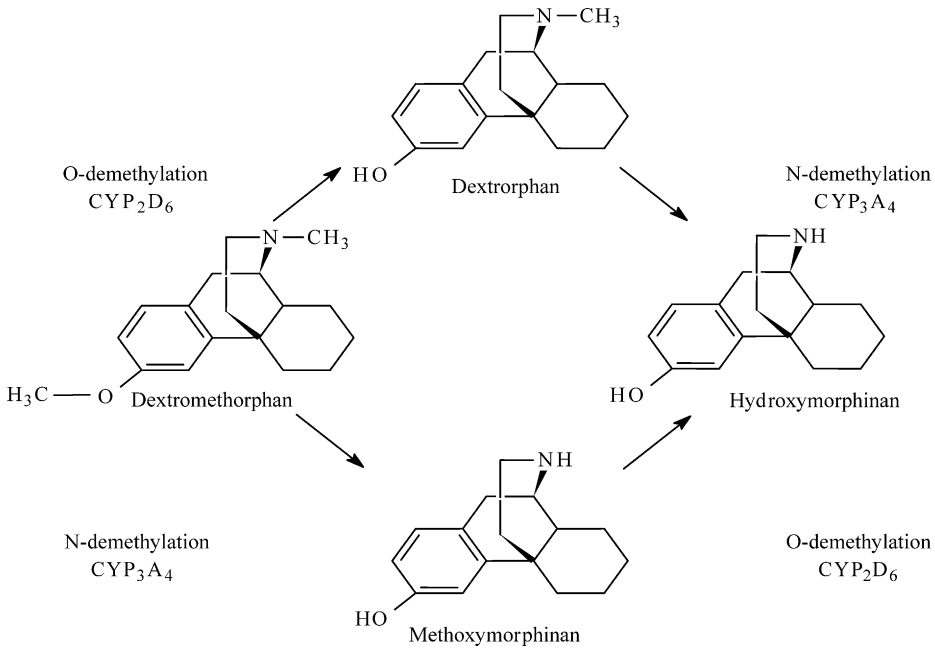

Fig. 1. Chemical structures and metabolic pathway of DM.

LLE but usually such equipment is rather expensive and may not be practical for all laboratories. LLE produces an emulsion and requires the use of large amount of highly-purity solvents, which are often hazardous and result in the production of toxic laboratory waste. Prior to the chromatographic analysis, when LLE and SPE are employed, there is a need for solvent evaporation, in order to preconcentrate the samples. Although SPE is less time-consuming than LLE, it still requires an appreciable amount of toxic solvent for analyte desorption.

Solid-phase microextraction (SPME) technique, initially introduced for analysis of volatile compounds, has gained an increasingly important role for isolation of vast varieties of compounds from aqueous media [18]. SPME overcomes the above difficulties by reducing or eliminating the use of organic solvents and by allowing sample extraction and preconcentration to be performed in a single run. The technology is more rapid and simple than the conventional methods. It is also inexpensive, portable and sensitive. In SPME, sorbent coated silica fibers are used to extract analytes from solid, aqueous or gaseous samples. After extraction, the fibers are directly transferred into the injection port of a gas chromatograph or special interface coupled to HPLC, via a modified syringe, after which the analytes are desorbed, thermally or by solvents, and subsequently analyzed [19–24,25].

SPME is predominantly performed on commercially available SPME fibers coated with different sorbents having various polarities [20]. The recommended operating temperatures for these fibers are generally within the range of 200–270 °C [26,27], which is not suitable for analysis of some polar compounds. Moreover, all these fibers are often prepared by physical deposition of the polymer coating on the surface of the fused silica fiber and that is the most likely reason for the low thermal and chemical stability. Sol–gel chemistry offers a simple and convenient pathway for the synthesis of advanced material systems to overcome this problem, by providing efficient incorporation of organic components into the inorganic polymeric structure in solution under quite mild thermal conditions. Clearly, creating chemical binding between the solid-phase coating and the substrate surface leads to more stable performance of the coating and subsequently extends the SPME application field toward less volatile and more polar compounds [28–31].

In the continuation of our research interests on trace determination of organic compounds in aqueous media [32–34], a simple, rapid, sensitive and reproducible SPME-GC-MS method for the determination of DM and DP in plasma is described. Three different coated fibers including poly(dimethylsiloxane) (PDMS), poly(ethylenepropyleneglycol) monobutyl ether (Ucon) and PEG were prepared, based on sol–gel technology, and evaluated for the SPME of these analytes from human plasma followed by GC-MS analysis.

2. Experimental

2.1. Chemicals and reagents

Tetramethoxysilane, poly(methylhydrosiloxane) (PMHS), trifluoroacetic acid (TFA), hexamethyldisilazane (HMDS), polyethylene glycol 4000 (PEG), methylene chloride, methanol, sodium hydroxide, hydrochloric acid, an-