

The Determination of Urinary Metabolites of Dextromethorphan by MEPS™-ESI-LCMS

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Introduction

Micro Extraction by Packed Sorbent (MEPS™) is an adaptation of SPE into a miniaturized device with a typical void volume of less than 10 µL. With operating volumes of this scale and its compatibility with autosampler syringes, MEPS™ allows the specificity of the solid-phase process to be harnessed for digital chromatography using discontinuous changes in solvent polarity. The eluant volumes are sufficiently small to be injected directly into a HPLC system and therefore permit the use of solid-phase extraction methodology in real time with the HPLC.

In this application, we describe the analysis of naturally voided human urine samples that were collected following the administration of single doses of dextromethorphan for both the parent drug and its urinary metabolites. The effectiveness of MEPS™ is compared with the same sample prepared off-line using conventional cartridge SPE. The method was used to demonstrate the effectiveness of MEPS™ for extraction of biological fluids for LCMS analysis.

Experimental

Robitussin DX Dry Cough Forte syrup (equivalent to 30 mg dextromethorphan hydrobromide, Wyeth Consumer Healthcare Pty Ltd, NSW, Australia) was administered orally to an 80 kg human male volunteer. Naturally voided urine samples were collected at 0, 2, 3 and 4 hours following administration and were stored frozen at -20 °C until required for analysis.

Typically, urine (3 mL) was diluted with 0.2 M sodium phosphate buffer (3 mL, pH 6.0) and the sample enzyme hydrolysed by incubation with 50 IU of glucuronidase enzyme (E.coli) at 50 °C for one hour. The specimen was centrifuged at 2000 g to remove suspended materials and the supernatant used for either conventional SPE or MEPS™ extraction. A 20 minute automated sample preparation by conventional SPE was performed as described previously [1]. MEPS™ extraction was performed on C18 MEPS™ BINS fitted to a 100 µL MEPS™ syringe. BINS were conditioned sequentially with methanol (50 µL) and water (100 µL). The sample (12 x 80 µL) was passed through the sorbent, followed by water (80 µL), saturated sodium tetraborate solution (50 µL), water (80 µL) and air (2 x 80 µL at 50 µL/sec). Retained analytes were eluted directly to an autosampler vial with methanol (2 x 20 µL), diluted with 40 µL of 0.1 % aqueous acetic acid for analysis. Extraction time was less than 5 minutes.

Samples were analyzed by HPLC on a Shimadzu Prominence LC-20 system (Kyoto, Japan) on a ProteCol-P C18 HQ105 (1500 mm x 4.6 mm ID, SGE Analytical Science, Melbourne, Australia) at temperature of 40 °C and a flowrate of 0.7 mL/min. The mobile phase was varied on a linear gradient from 1 % aqueous acetic acid in 10 % methanol to 1 % aq. acetic acid in 90 % methanol over 10 minutes then held isocratically for a further 10 minutes. Detection was by positive ion ESI-MS using a LCQ Classic MS (Thermo, San Jose, USA). MS operating conditions are available on request. Speculative structural elucidation of metabolites was made on the basis of mass fragmentography using MS³ or MS⁴ as required.

Results and Discussion

The SIM chromatogram for urine extracts prepared by MEPS™ and conventional SPE are shown in Figure 1. The chromatograms for both techniques were qualitatively and quantitatively similar with the conventional technique validating the MEPS™ approach to analysis for LCMS detection. The detection of additional late eluting peaks in the MEPS™ chromatogram reflects the increased specificity of the mixed-mode conventional SPE approach over the faster reversed-phase MEPS™ approach.

On the basis of previous studies [2], fragmentation of the protonated analytes is dominated by neutral loss of the amine moiety and the D-ring (Figure 2) to give characteristic ions that are shifted in mass by metabolic modification. For a selected, protonated molecular ion, shifts in mass of fragment ions may be used to deduce tentative structures. The shift in retention time for proposed metabolites may also be correlated with structural modification. Reversed-phase retention of dextromethorphan is due to the whole molecular surface excluding the region around the protonated amine (Figure 3). Metabolic modification by O-demethylation or hydroxylation of the aromatic ring decreases the hydrophobicity of this portion of the molecule and makes a significant change to the available molecular surface for reversed phase interaction leading to a reduction in retention time.

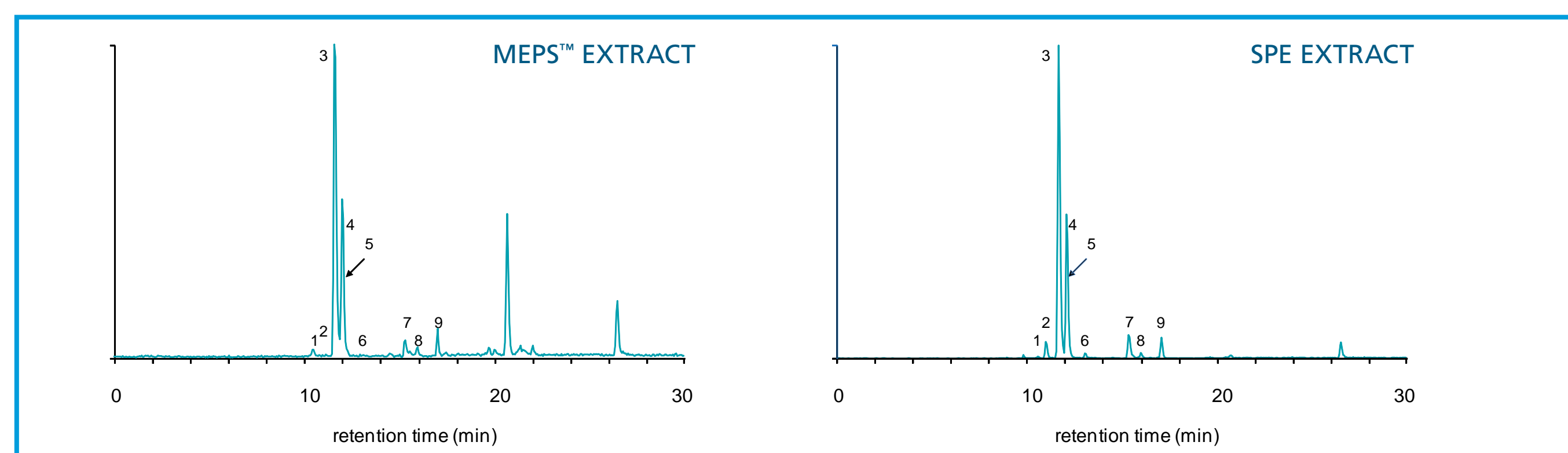


Figure 1: The SIM chromatograms for dextromethorphan positive sample extracted using MEPS™ (top) and conventional mixed-mode SPE (bottom).

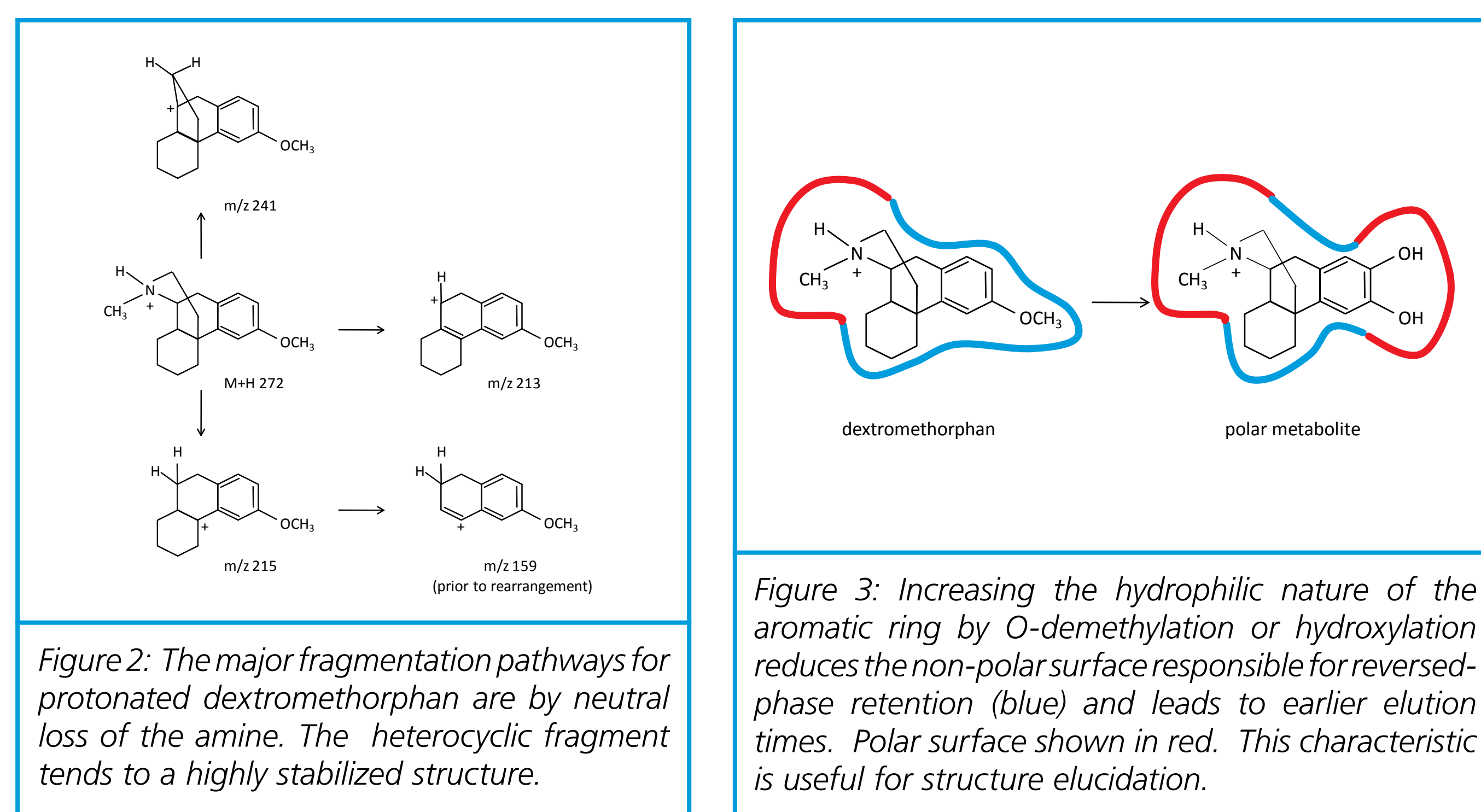


Figure 2: The major fragmentation pathways for protonated dextromethorphan are by neutral loss of the amine. The heterocyclic fragment tends to a highly stabilized structure.

Figure 3: Increasing the hydrophilic nature of the aromatic ring by O-demethylation or hydroxylation reduces the non-polar surface responsible for reversed-phase retention (blue) and leads to earlier elution times. Polar surface shown in red. This characteristic is useful for structure elucidation.

Using these principles, 8 metabolites and the unchanged parent were identified (Figure 4). Routes of metabolism include O-demethylation, N-demethylation and aliphatic hydroxylation on the D-ring. It is not yet clear if the dominant m/z 199 fragment observed for the N-desmethyl metabolite is due to an unexpected loss or the contamination of the spectrum by the co-eluting internal standard (acepromazine M+H 327).

Conclusion

We report here a rapid sample preparation using MEPS™ and an ESI-LCMSn method for the detection of dextromethorphan metabolites in urine. The extraction time using MEPS™ was less than five minutes per sample while extraction time of the same specimen using conventional SPE was 20 minutes.

Five major species were detected and determined to be the unchanged parent and the O-desmethyl-, N,O-didesmethyl-, hydroxy-O-desmethyl and N-desmethyl metabolites. Tentative evidence of a further 4 minor metabolites derived from ring hydroxylation and side chain oxidation was also found.

References

- [1] Wynne PM, Batty DC, Vine JH and Simpson NKJ, Chromatographia, 59 (4/5), S50-S61, (2004).
- [2] Strife RJ, Robosky LC, Garrett G, Ketcha MM, Schaffer JD and Zhang N, Rapid Comm. Mass. Spec., 14, 250-260, (2000).

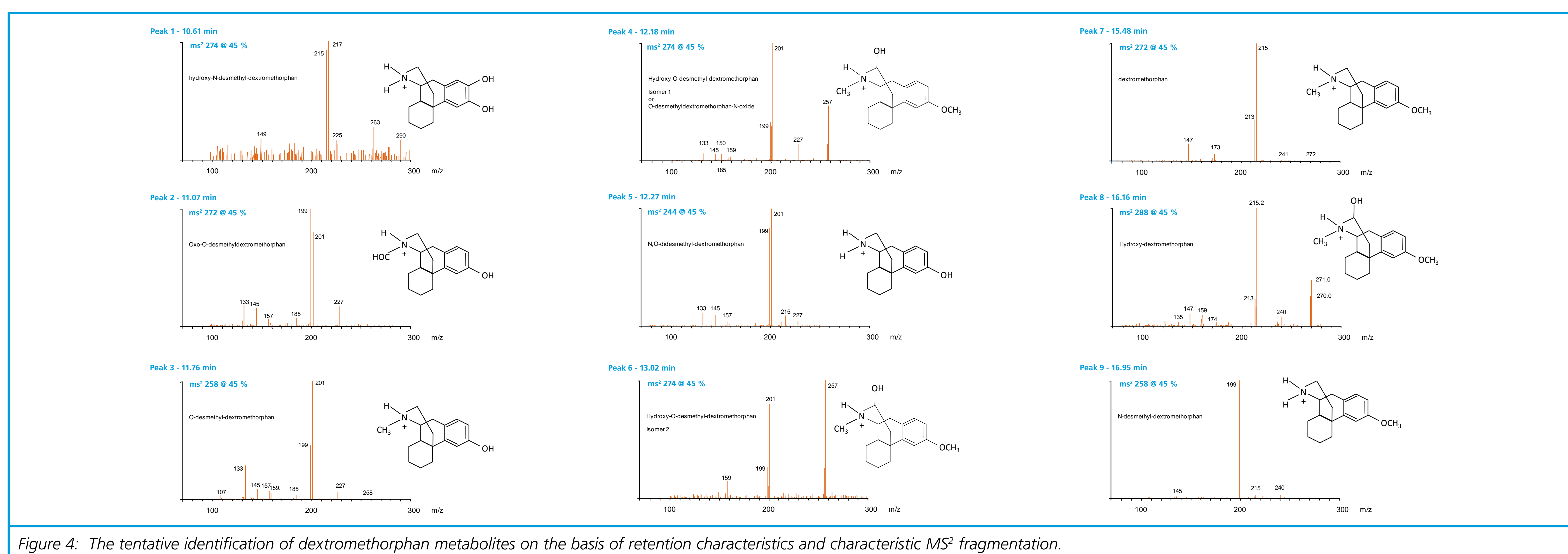


Figure 4: The tentative identification of dextromethorphan metabolites on the basis of retention characteristics and characteristic MS² fragmentation.

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