Identification of Dextromethorphan Metabolites in Urine

Application Note

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Introduction

When administered to individuals, drugs generally undergo biological processing (metabolism) into variants that are readily excreted from the body. The type of metabolites formed and rate of excretion are a function of the enzyme activity of the individual, with around half of all drugs being cleared by enzymes of the cytochrome P450 family[1]. Many drugs are metabolized extensively or exclusively by a particular isoform of this polymorphic family. The CYP3A4 and the CYP2D6 families of cytochrome P450 enzymes are considered the most important in this activity.

Dextromethorphan is commonly used as a probe for the activity of the CYP2D6 metabolic phenotype. Subjects are typically "poor metabolizers" with large amounts of unmodified dextromethorphan being excreted in the urine, or "extensive metabolizers" who excrete the drug primarily in the form of two glucuronide conjugates with essentially no unmodified dextromethorphan present[2]. There is also a class of metabolizers who have multiple copies of the gene that produces the CYP2D6 enzymes, and these individuals metabolize many drugs at such high rates as to make these drugs therapeutically ineffective[1].

Dextromethorphan has significant side effects when used in high dosage; these side effects include euphoria, hallucinations, and visual disturbances, making it a candidate for drug abuse. Its reputation as having effects similar to methylenedioxymethamphetamine ("Ecstasy") has led to a dramatic increase in dextromethorphan abuse[3]. The identification of dextromethorphan metabolites is shown here as an illustrative example of the use of the Agilent 1100 Series LC/MSD Trap XCT Plus system and ACD/Labs version 8 software in metabolism or forensic studies.

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Experimental

The samples were analyzed using an Agilent LC/MS system consisting of a wellplate sampler with thermostat, binary pump, thermostatted column compartment, and Agilent 1100 Series LC/MSD XCT Plus ion trap mass spectrometer.

Samples were prepared as follows. Urine for use as a control was collected from a healthy male volunteer. Then a single dose of an over-the-counter anti-tussive preparation containing 30 mg dextromethorphan was administered. A pooled urine sample was collected over next 0–6 hours, immediately acidified using neat phosphoric acid (1 mL acid per 100 mL urine), and frozen at –20°C until needed. For analysis, an aliquot of the sample and control was thawed, mixed with an equal volume of acetonitrile, centrifuged (10,000 g, 10 min), and the supernatant injected into the LC/MSD system.

Results and Discussion

Metabolites were located by comparison of the sample and control to find all significant differences. The Compare LCMS operation of the ACD/Labs software package did this automatically, returning a set of mass chromatograms indicating the unique differences as defined by the parameters used for the comparison. Figure 1 displays the results of this comparison, showing that at least three components of the sample (Figure 1, top window) were unique. Three of the top-ranked mass chromatograms had *m/z* values of 258, 420, and 434, corresponding to precursor ions for three known common metabolites of dextromethorphan[4–6].

INSTRUMENT CONDITIONS

LC Conditions

Column:	ZORBAX SB-Aq, 50 x 2.1 mm, 3.5 µm (part number 871700-914)
Column temperature:	35 °C
Mobile phase:	A = 0.1% formic acid in water B = 0.1% formic acid in acetonitrile
Gradient:	0% B at 2 min 50% B at 15 min stop run at 20 min post run 5 min
Flow rate:	0.4 mL/min
Injection volume:	1 µL
WPS temperature:	4 °C

MS Conditions

Ionization mode:	Positive ESI
Drying gas flow:	12 L/min
Nebulizer:	50 psig
Drying gas temperature:	350 °C
Vcap:	–2500 V
Skim 1:	40 V
Capillary exit:	100 V
Trap drive:	40
Scan mode:	Ultrascan
Scan range:	100–700 *m/z*
Averages:	4
ICC:	On
Max. accumulation time:	100 ms
Target:	500000
Automatic MS/MS:	
Number of parents:	3
Isolation width:	4 *m/z*
Fragmentation amplitude:	1.1 V
Active exclusion:	On
Exclude after no. of spectra:	2
Release after:	0.5 min
Smart Frag:	On, 30–200%

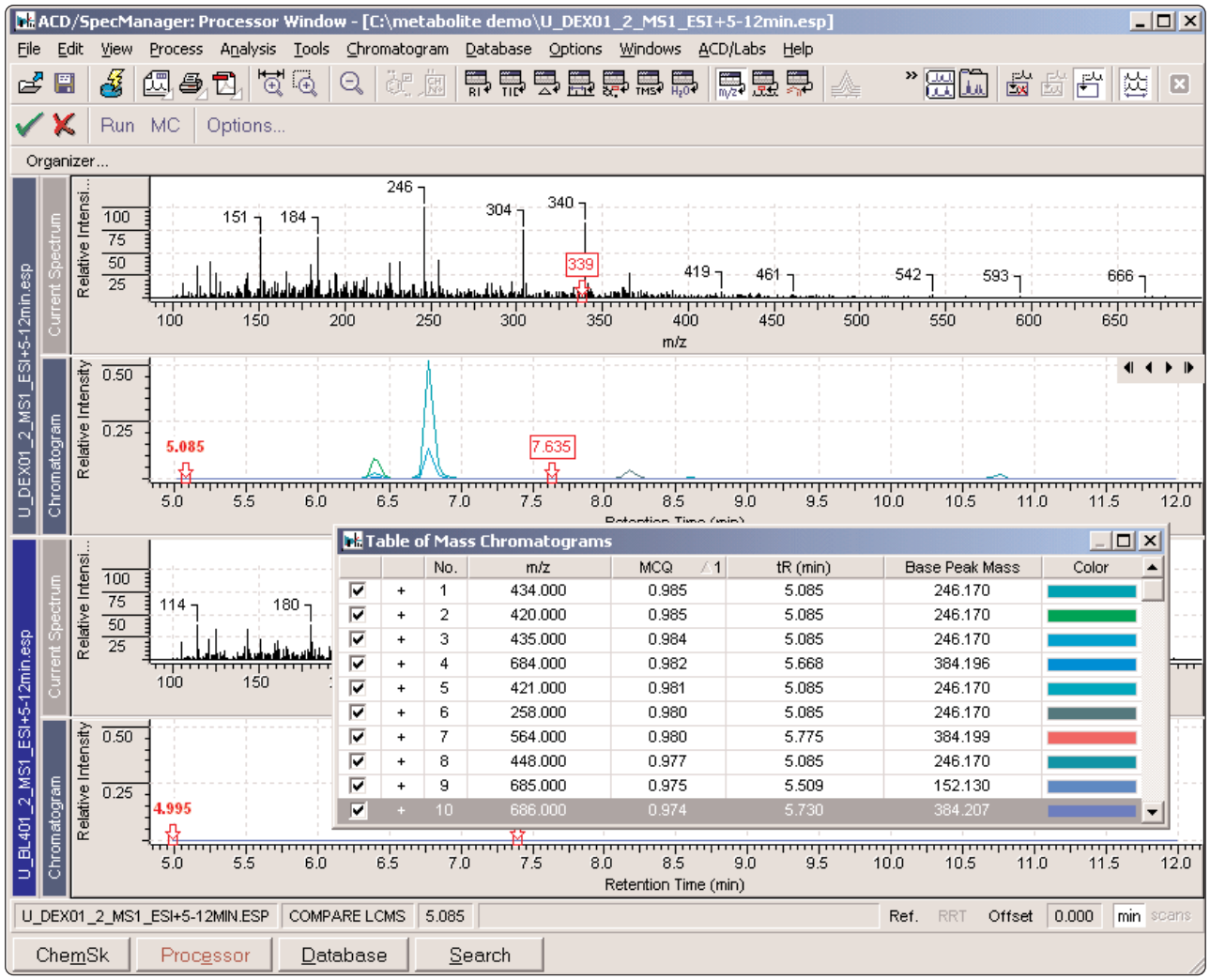

Figure 1. Comparison of sample and control for urine samples after dosage with dextromethorphan

Even though some endogenous compounds were also present in both the sample and control, the Compare LCMS operation determined that their amounts did not change significantly, and thus they did not appear in the list of mass chromatograms. This is illustrated in Figure 2, where the endogenous component at a retention time of 6.2 minutes was ignored as being a candidate for further investigation.

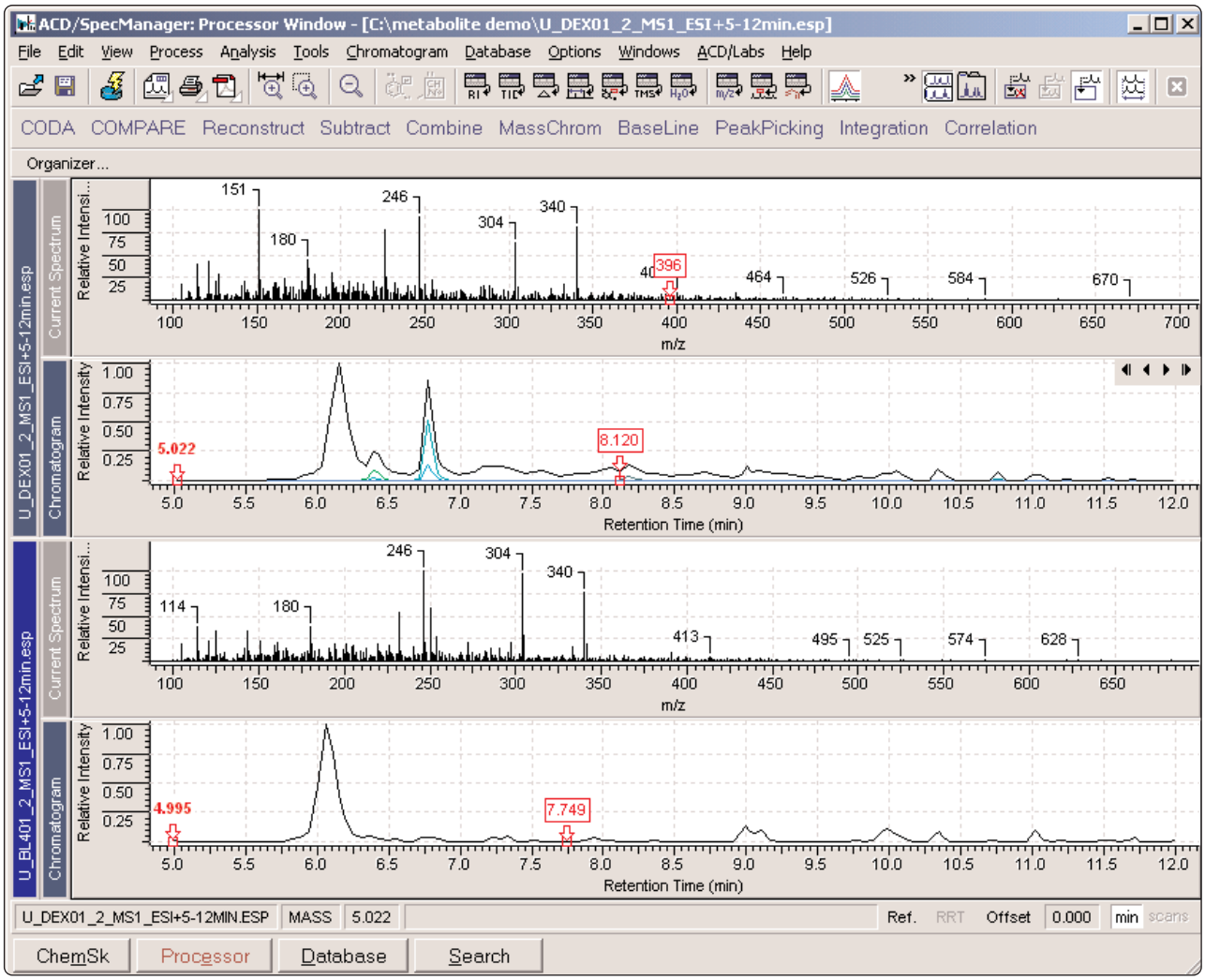

Figure 2. Comparison of sample and control for urine samples after dosage with dextromethorphan. The TIC in both the sample and control (black traces) shows a large endogenous component present at about 6.2 minutes, but no mass chromatogram (colored traces) has a maximum at this time, indicating there is no significant difference between sample and control for this component.

Assignment of fragments of the chemical structure to peaks in an MS/MS spectrum was performed by the AutoAssignment process, as is shown here for one of the metabolites, the glucuronide conjugate of 3-hydroxymorphinan.

Typically, the vast majority of the abundance in the mass spectrum was assigned automatically (in this case, 74%). If needed, fragment assignments may be added to these results manually as well.

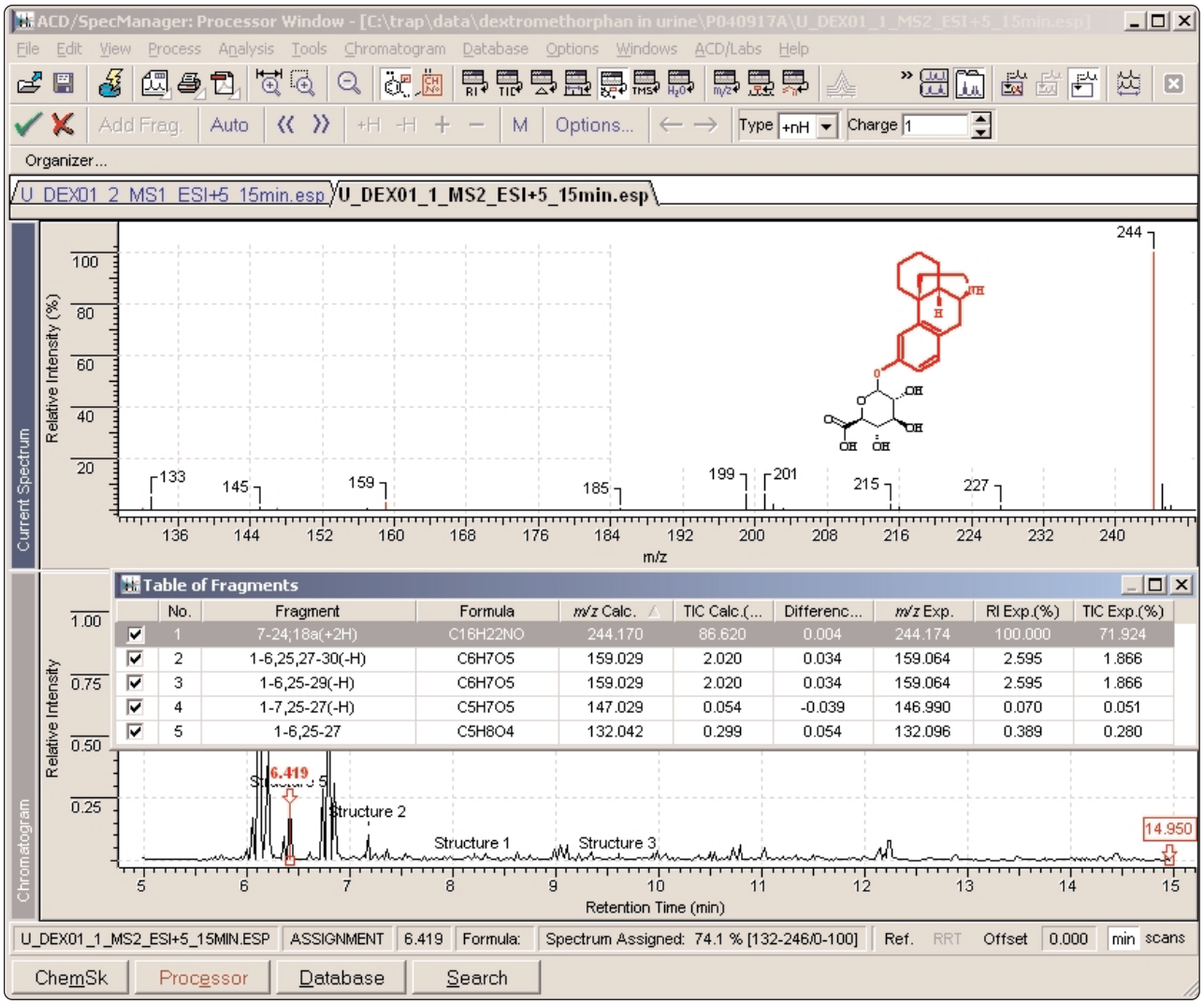

Figure 3. AutoAssignment of a chemical structure and fragments to an MS/MS spectrum

Once this assignment was made, the MS/MS and MS spectra corresponding to this compound were archived in a database for future reference and searching. Relevant information such as Auto-Assignment values was automatically stored with the data. The database acts as an electronic notebook for analytical data.

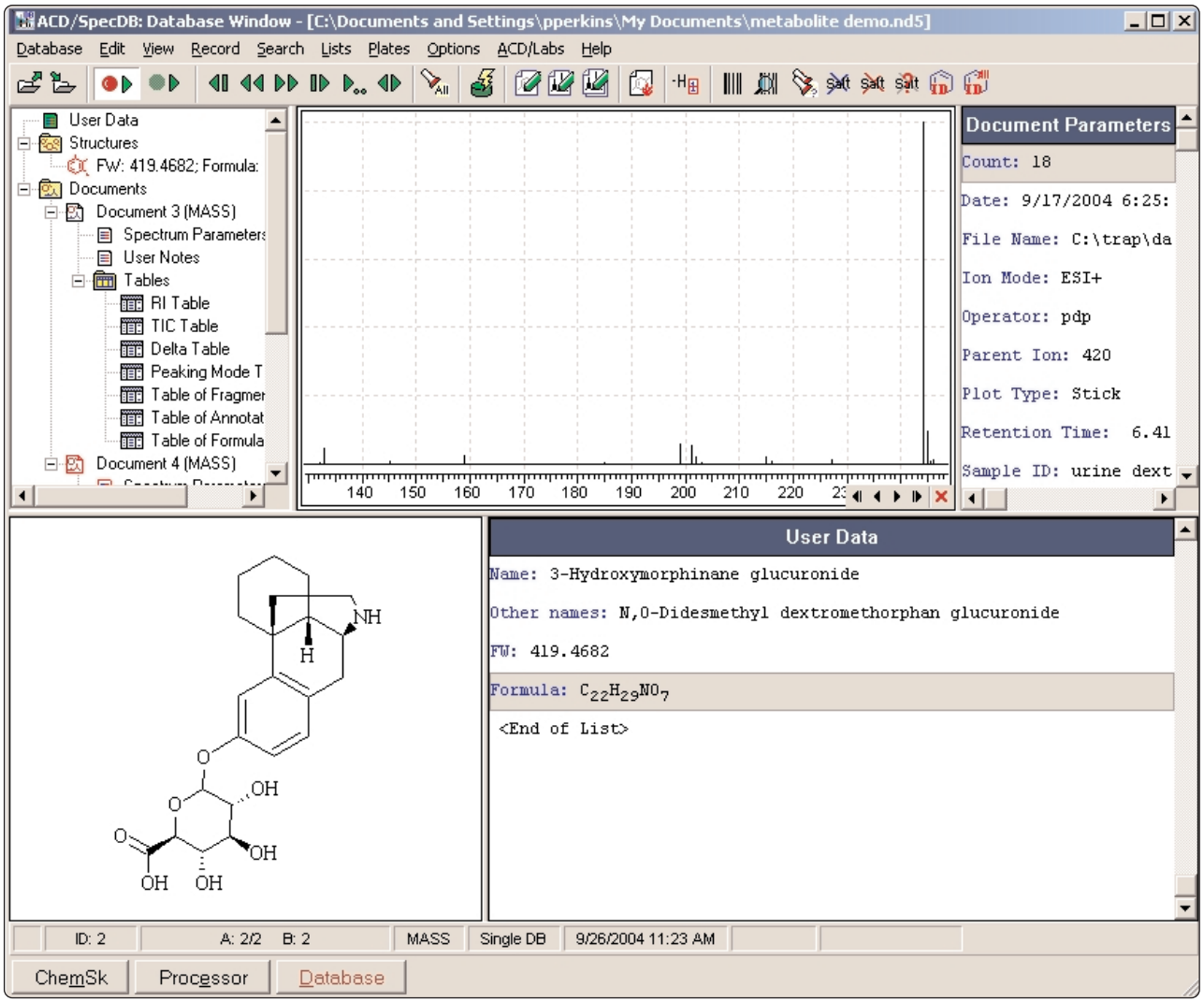

Figure 4. Archival of analytical information for one of the compounds identified in this work

Three major metabolites of dextromethorphan were confirmed to be present: dextrorphan (parent *m/z* = 254), dextrorphan glucuronide (parent *m/z* = 434), and 3-hydroxymorphinan glucuronide (parent *m/z* = 420). In addition, the known metabolite 3-hydroxymorphinan (parent *m/z* = 244) was found in small amounts. A minor metabolite consistent with hydroxydextromethorphan (parent *m/z* = 288) was tentatively identified, as well as traces of unmetabolized dextromethorphan (parent *m/z* = 272). The results of the analysis indicate that this person fits the profile of an "extensive metabolizer" of dextromethorphan, with the vast majority of the drug being excreted in the urine in the form of glucuronide conjugates.

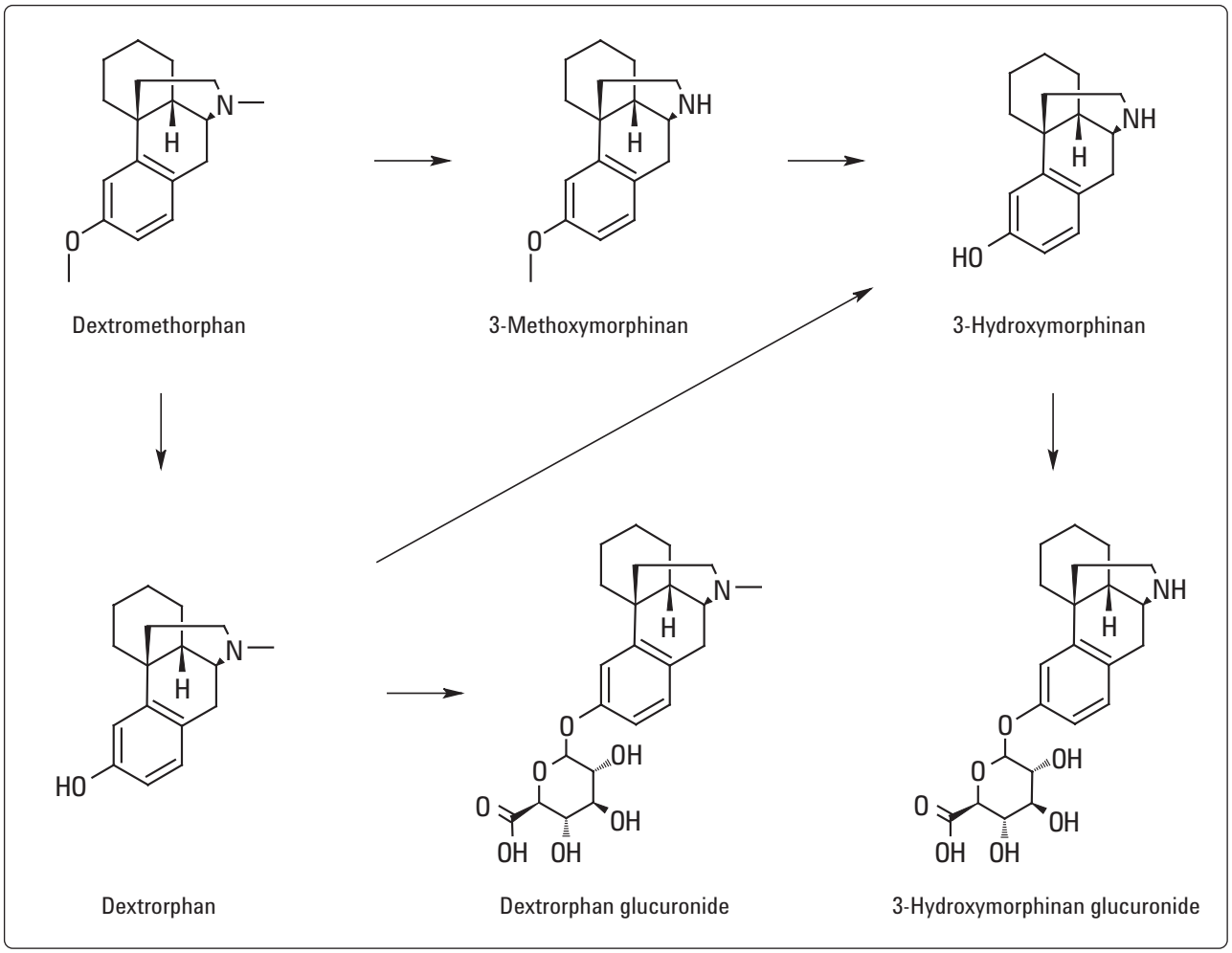

Figure 5. Biotransformation map for dextromethorphan. All metabolites shown with the exception of 3-methoxymorphinan were found in this study.

Conclusions

The Agilent 1100 Series LC/MSD Trap XCT Plus system provides rapid and accurate MS^n data to be used for structural identification of metabolites in samples. The ACD/Labs version 8 software has tools to quickly locate differences between samples and controls, draw structures, assign fragmentation information to mass spectral features, and archive the results in a retrievable manner. The combination of the two provides a powerful means of analyzing drugs and their metabolites in biological matrices such as urine.

References

1. The Worldwide Physiologist: cytochrome P450; **http://www.anaesthetist.com/physiol/basics/metabol/cyp/cyp.htm#iso.**
2. Lutz, U.; Volkel, W.; Lutz, R.W.; Lutz, W.K.; *Naunyn-Schmied. Arch. Pharmacol.,* **2004,** *369 Suppl. 1,* R155.
3. McFee, R.B.; Mofenson, H.C.; Caraccio, T.R.; *Arch. Fam. Med.,* **2000,** *9,* 123.
4. Bendriss, E.-K.; Markoglou, N.; Wainer, I.W.; *J. Chrom. B,* **2001,** *754,* 209–215.
5. Hendrickson, H. P.; Gurley, B. J.; Wessigner, W. D.; *J. Chrom. B,* **2003,** *788,* 261–268.
6. Afshar, M.; Rouini, M.-R.; Amini, M.; *J. Chrom. B,* **2004,** *802,* 317–322.

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