Prediction of metabolite mass spectra (MS) of papaverine for library matching using the MS^n capabilities of ion trap LCMS

Richard Evers, Toxicology Unit, King's College Hospital, Denmark Hill, London SE5 9RS

Introduction

Papaverine is an alkaloid present in the opium poppy, Papaver somniferum, that survives the illicit manufacture of heroin from opium. Papaverine is O-demethylated to hydroxypapaverine and to dihydroxypapaverine, and subsequently glucuronidated, and these metabolites are excreted in the urine of illicit heroin users. Reference materials and mass spectra are not routinely available for these metabolites. Ion trap mass spectrometers can selectively isolate and fragment ions in MSn collision events to produce reference spectra. Adjusting the collision energy and the product ion selected at each stage can reproduce the metabolism of the papaverine stage by stage to enable spectra to be acquired.

Aims

The aim of this work was to produce library spectra of hydroxypapaverine, dihydroxypapaverine and their glucuronide metabolites to identify use of illicit heroin in drug users taking prescription diamorphine.

Methods

A 100 mg/L solution of papaverine (Sigma-Aldrich) was infused into an LCQ fleet ion-trap mass spectrometer (Thermo Fisher Scientific) using the integral syringe driver (5 µL/min) with an electrospray ionisation source. The collision energy to fragment papaverine ($[M+H]^+$ m/z = 340) [Figure 1a] was adjusted to give optimal recovery of the $[M-CH2]^+$ ion (m/z 325) [Figure 1b]. This ion was further fragmented, with the second stage collision energy optimised to minimise the $[M-CH2]^+$ ion and produce a characteristic MS(3) spectrum [Figure 1c]. The $[M-CH2]^+$ ion was separately fragmented, optimising the collision energy for isolation of the $[(M-CH2)-CH2]^+$ ion (m/z 310), corresponding to the dihydroxypapaverine metabolite. This ion was isolated and a third collision event optimised to produce an MS(4) spectrum. The acquired spectra were saved to in-house MS(2) and MS(3) mass spectral libraries. These library spectra were validated by analysing hydrolysed samples (1000 units β-glucuronidase (*Helix aspersa*) in 0.75 mL 1 mol/L pH 5.0 acetate buffer) previously shown to be positive for papaverine metabolites by GC-MS and confirming a good library match. The retention times of these metabolites were recorded by LC-MS analysis (X-LC, Jasco) with a multi-step gradient of 1 % (v/v) formic acid / water with 10 mmol/L ammonium formate and 1 % (v/v) formic acid / acetonitrile on a 2.1 x 50 mm 5 µm Hypersil PFP Gold column (Thermo Fisher Scientific). The same samples were analysed without hydrolysis by monitoring the glucuronide metabolite parent ions (m/z 502 for hydroxypapaverine [Figure 2a-c] and m/z 488 for dihydroxypapaverine [Figure 3a-c]) using MS(3). Matching these spectra to the library allowed capture of the MS(2) spectra and retention times of the glucuronides.

Papaverine Infusion

Figure 1a

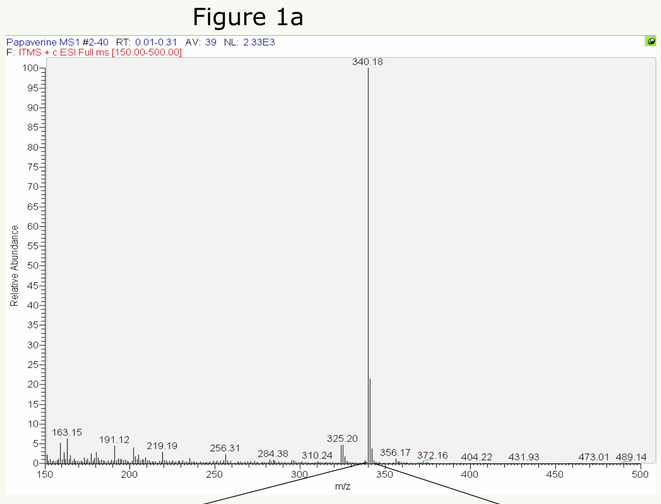


Figure 1b

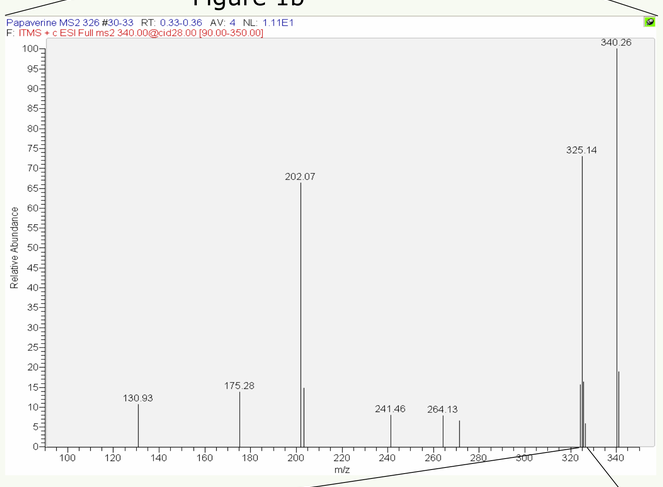


Figure 1c

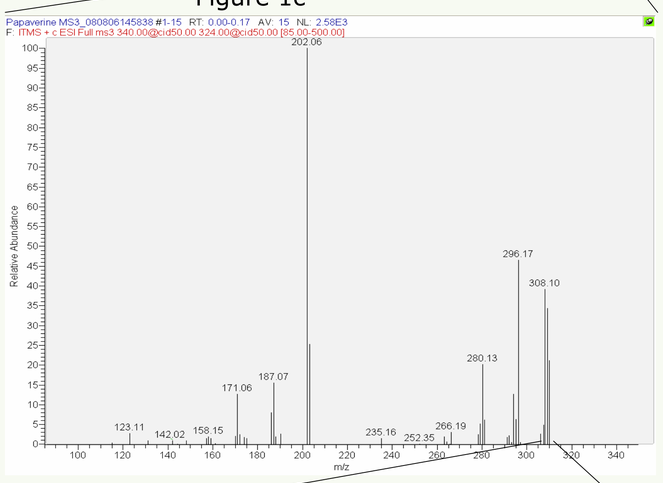
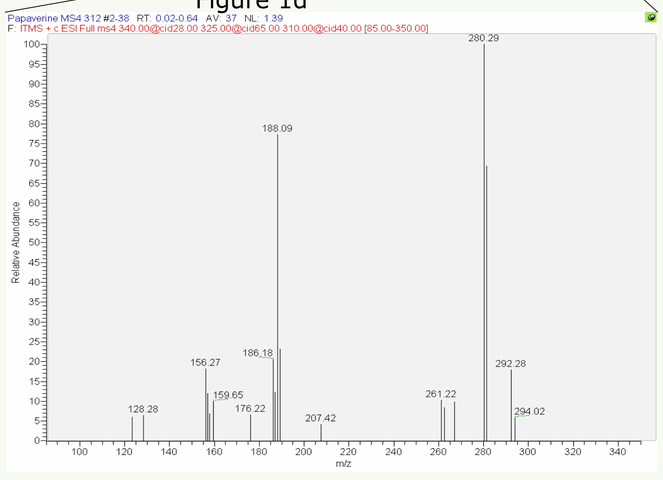
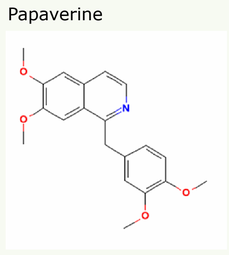


Figure 1d



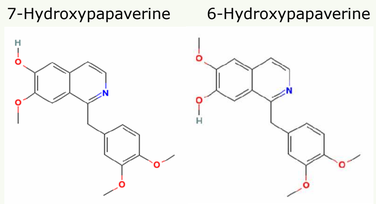
Tune compound—Papaverine
ESI Voltage 3.5 kV
Capillary Temperature 300°C
Sheath Gas Flow 0
Isolation Width 4.0
Scan Range 150—500

Papaverine



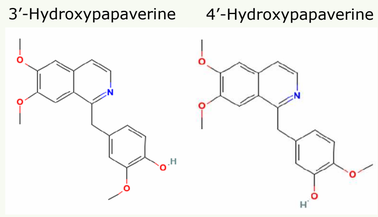
$C_{20}H_{21}NO_4$
M_r=339

7-Hydroxypapaverine 6-Hydroxypapaverine

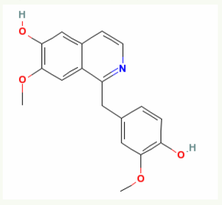


$C_{19}H_{19}NO_4$
M_r=325

3′-Hydroxypapaverine 4′-Hydroxypapaverine



4′,6-Dihydroxypapaverine



$C_{18}H_{17}NO_4$
M_r=312

Metabolites in Urine

Figure 2a - Hydroxypapaverine-glucuronide

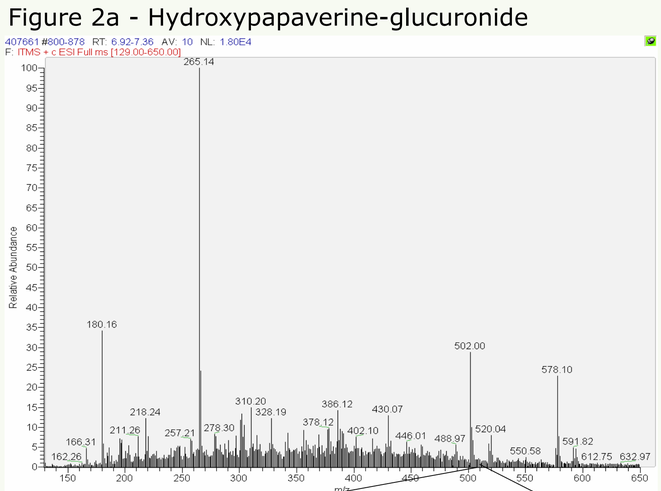


Figure 2b

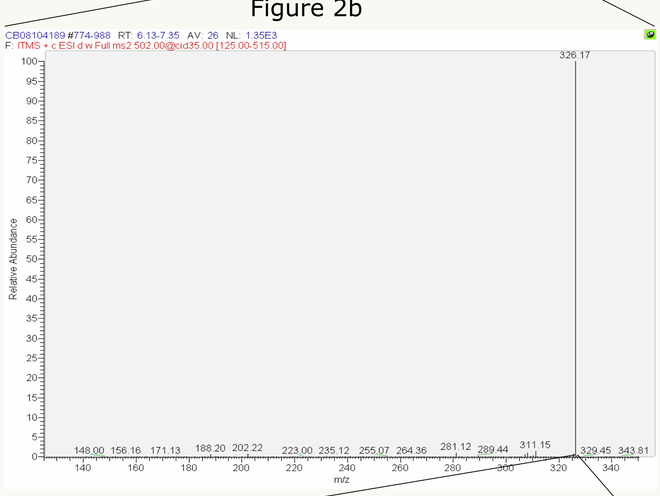


Figure 2c

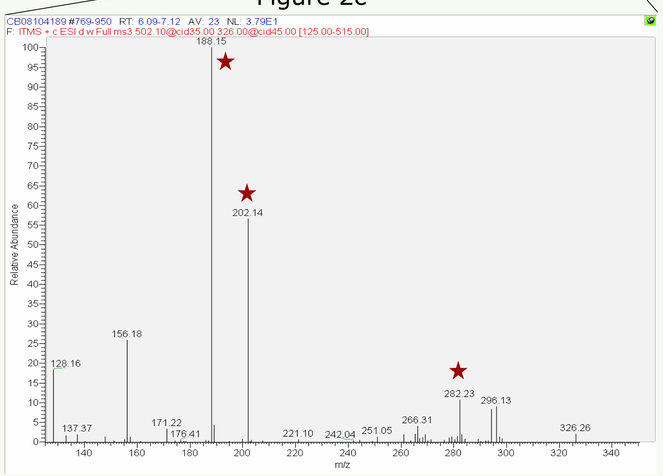


Figure 3a - Dihydroxypapaverine-glucuronide

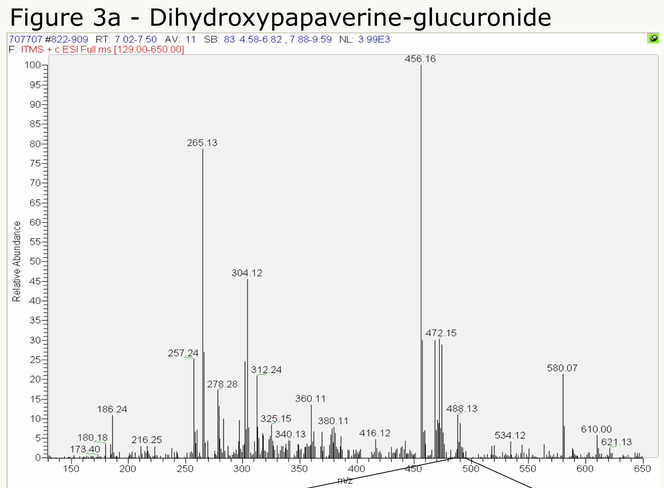


Figure 3b

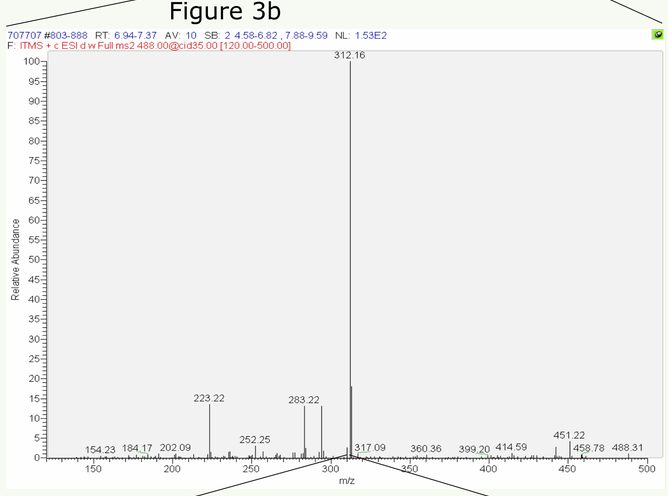
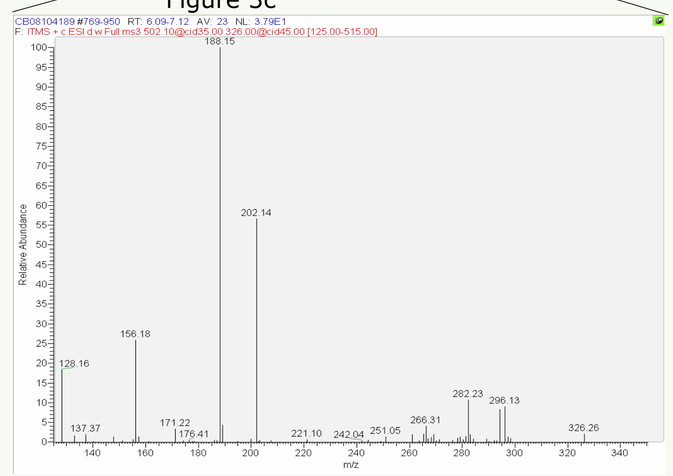


Figure 3c



Wideband activation causes fragmentation of parent ions and any daughter ion with a mass difference of up to 20 mass units below the parent ion. The intensity of m/z 308 and m/z 296 fragments are therefore much reduced compared to the spectra displayed in figure 1.

Daughter ion structures

Structures (★ - Figure 2c) predicted from theory using HighChem Mass Frontier 5.1 fragmentation library

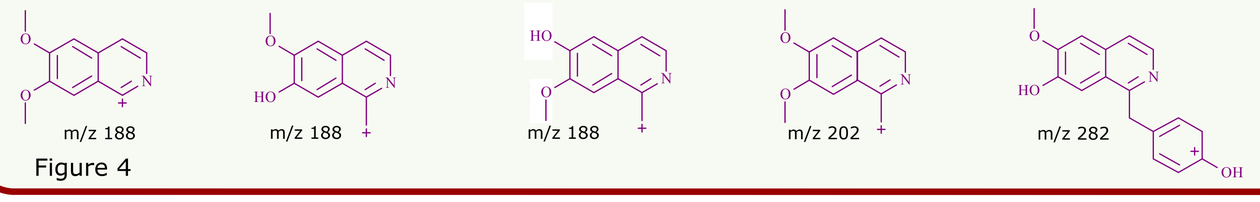


Figure 4

Results

Reference spectra were produced for hydroxypapaverine, dihydroxypapaverine and their glucuronides, and used in an automated urine drug screening method. These metabolites were detected in urine after use of illicit heroin, but not after pharmaceutical diamorphine medication.

Conclusion

The MSn capability of an ion trap has enabled the mass spectra of papaverine metabolites to be recorded. These spectra were used to identify illicit heroin use in patients prescribed diamorphine.

KingsPath Clinical Diagnostic Pathology Services
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