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Buprenorphine by GC-MS after derivatization by direct extractive alkylation

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Introduction

Buprenorphine is a narcotic analgesic and is administered in low doses (0.2-0.6mg) by sublingual or intravenous means. It is metabolised by N-dealkylation to norbuprenorphine and is excreted into the urine primarily as glucuronide conjugates.
Several GC procedures have been reported for the determination of buprenorphine and norbuprenorphine [1-4]. This communication describes a GC-MS method for the determination of urinary buprenorphine and norbuprenorphine as their methyl derivatives after enzyme hydrolysis and derivatization by direct extractive alkylation.

Experimental

Instrumentation

Gas chromatograph	HP 5890 Series II
Detector	HP 5970B MSD
m/z for methylated buprenorphine	366, 380, 392, 424, 448, 481
m/z for methylated norbuprenorphine	310, 326, 340, 352, 366, 384, 394, 441
Column	17m HP Ultra 2
Injector temperature	260°C
Detector temperature	290°C
Initial column temperature	231°C
Final column temperature	300°C
Temperature ramp	10°C/min to 300°C

Hydrolysis

To aliquots of urine (2ml) were added 150ml of 2M acetate buffer (pH 5), and 25ml of Type II b-glucuronidase from Helix Pomatia. The urine was hydrolysed in a water bath at 50°C for three hours and then allowed to cool to room temperature.

Direct extractive alkylation

To the hydrolysed samples were added 300ml of 3M sodium hydroxide, 150ml of 0.2M tetrahexylammonium hydrogen sulphate (prepared by dissolving 4.5g of the salt in 50ml of 0.5M sodium hydroxide) and 5ml of 0.3M iodomethane in toluene. The two phases were mixed at 25°C for 40-50 minutes and then centrifuged at 1500g for 5 minutes. To remove the co-extracted tetrahexylammonium salts the toluene phases were passed through 2.5-3.0cm columns of SM-7 resin, collected in disposable test tubes and evaporated to dryness under a stream of nitrogen at 35°C. The residues were reconstituted in 100ml of ethyl acetate before injection of a 3ml aliquot into the GC-MS system.

Results and Discussions

Mass spectra

Figure 1 illustrates the full scan electron impact mass spectrum of the methyl derivatives of buprenorphine and norbuprenorphine. The two mass spectra show similar fragmentation patterns (see Table 1).

TABLE 1 Principal fragment ions in the mass spectrum of the O-methyl ether derivative of buprenorphine and the N,O-dimethyl derivative of norbuprenorphine.

m/z O-methyl buprenorphine	m/z N,O-dimethyl norbuprenorphine	Origin
481	441	M^+
392	352	loss of CH_3OH and C_4H_9
424	384	loss of C_4H_9
380	340	loss of $C_6H_{13}O$

Urinary concentrations

After the sublingual administration of a 0.2mg dose of buprenorphine to a volunteer the method outlined under Experimental was applied to the determination of the urinary concentrations of buprenorphine and norbuprenorphine. The time courses for the urinary concentrations are illustrated in Figure 2. Buprenorphine was found in the urine from one hour after administration with the maximum concentration of 9.6ng/ml been reached two hours after administration. Norbuprenorphine was found in the urine from two hours after administration. Both

buprenorphine and norbuprenorphine were still detectable nineteen hours after the administration of a single dose. Figures 3 and 4 illustrate the selected ion traces for buprenorphine (m/z= 392, 380, 424) and norbuprenorphine (m/z= 352, 408, 384) at T=O (blank), 3.9 and 19.0 hours after the administration of a single 0.2mg dose of buprenorphine. Figures 3D and 4D illustrate the ion traces for a urine sample spiked to 20ng/ml with buprenorphine and norbuprenorphine.

Conclusions

The results demonstrate the usefulness of extractive alkylation for the determination of buprenorphine and norbuprenorphine in urine. The technique has been extended in the Sydney laboratory in order to screen for pholedrine, etilefrine, ritalinic acid (metabolite of methylphenidate), bamethan, ethamivan, pemoline, p-hydroxy metabolite of mesocarb, benzoylecgonine (metabolite of cocaine), morphine and nalbuphine.

References

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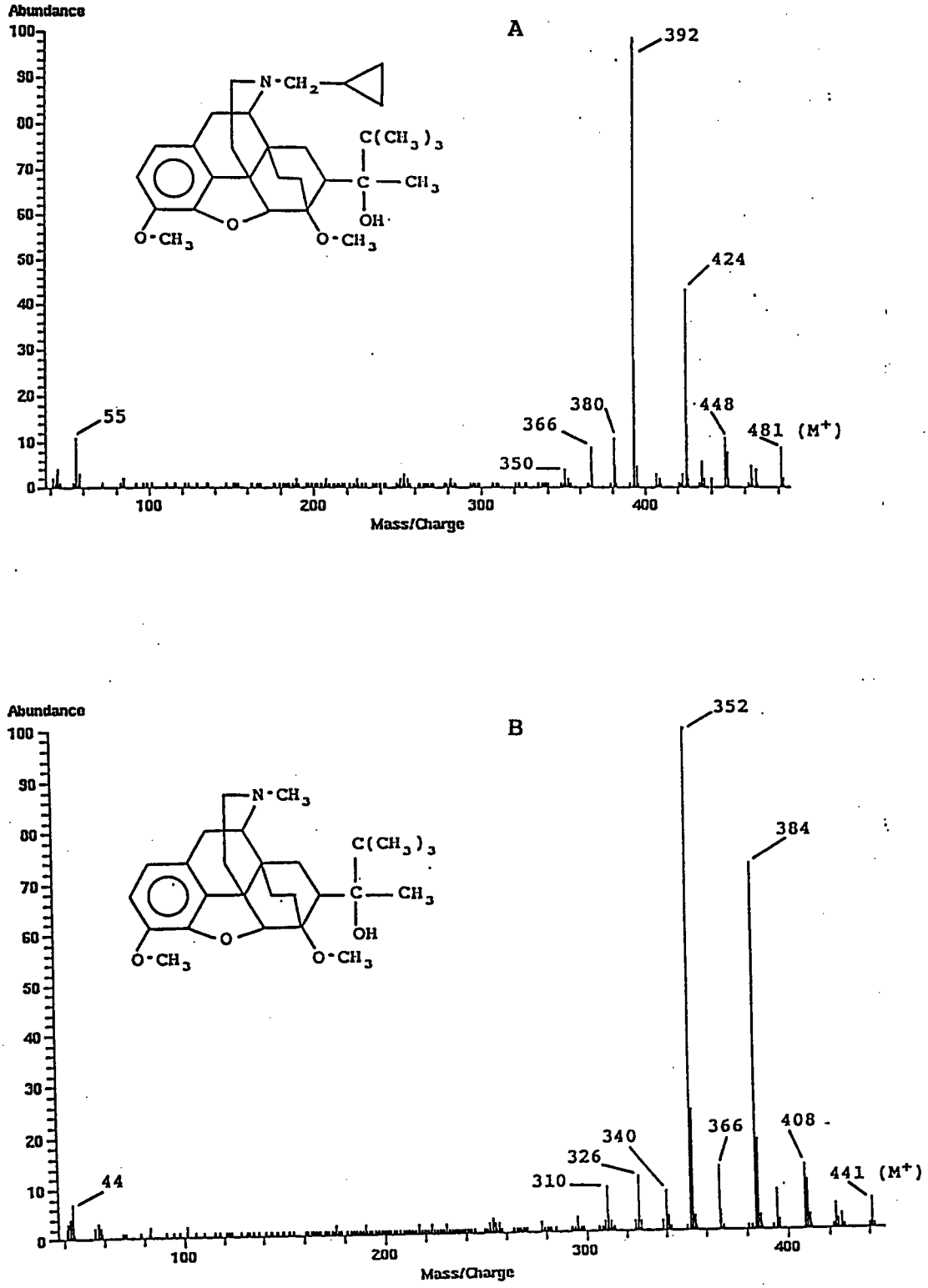

Figure 1. Full scan electron impact mass spectra of (A) the O-methyl ether derivative of buprenorphine and (B) the N,O-dimethyl derivative of norbuprenorphine

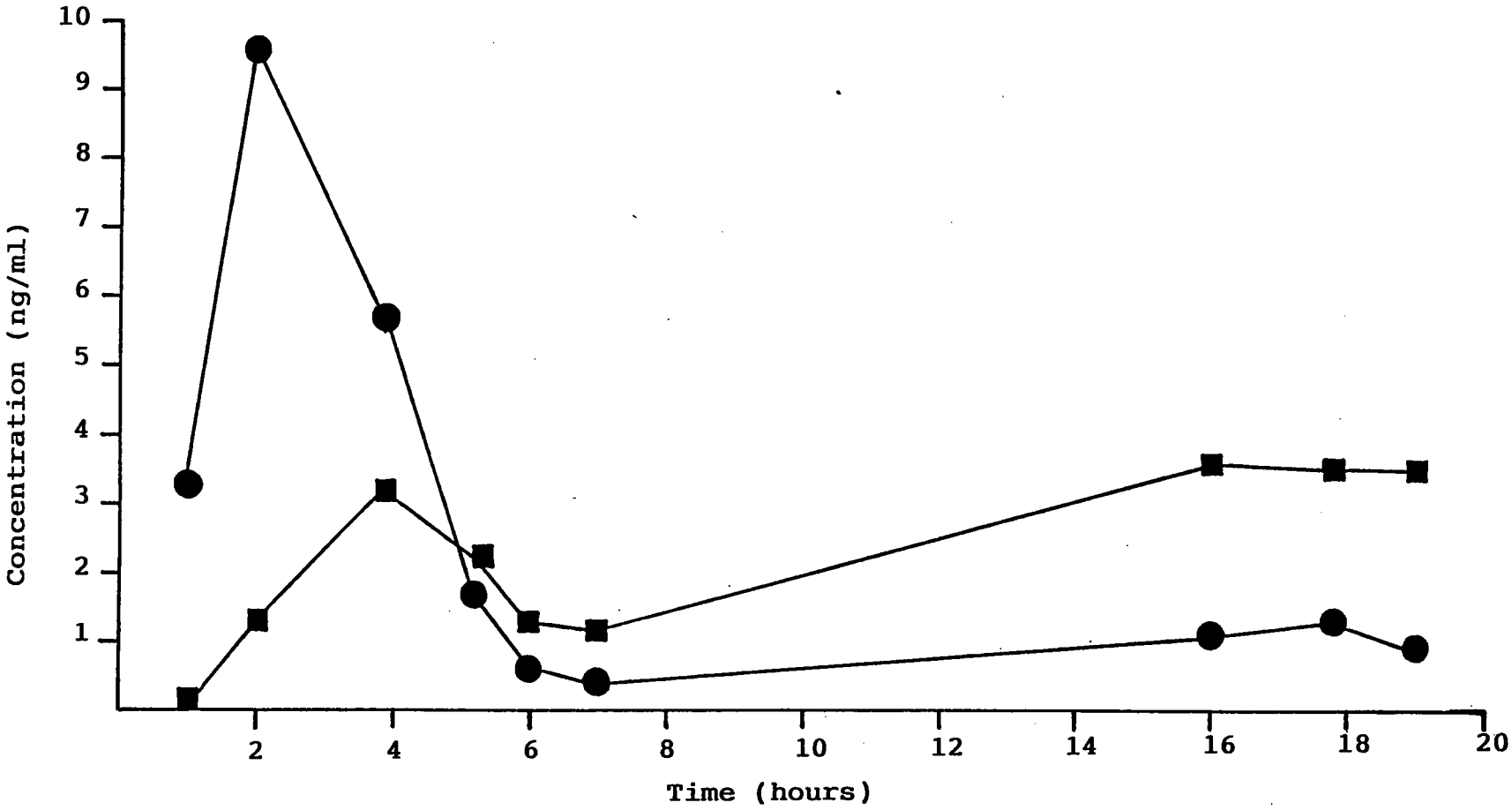

Figure 2. Time-courses for the urinary concentrations of buprenorphine (●) and norbuprenorphine (■) after the sublingual administration of a 0.2mg dose of buprenorphine

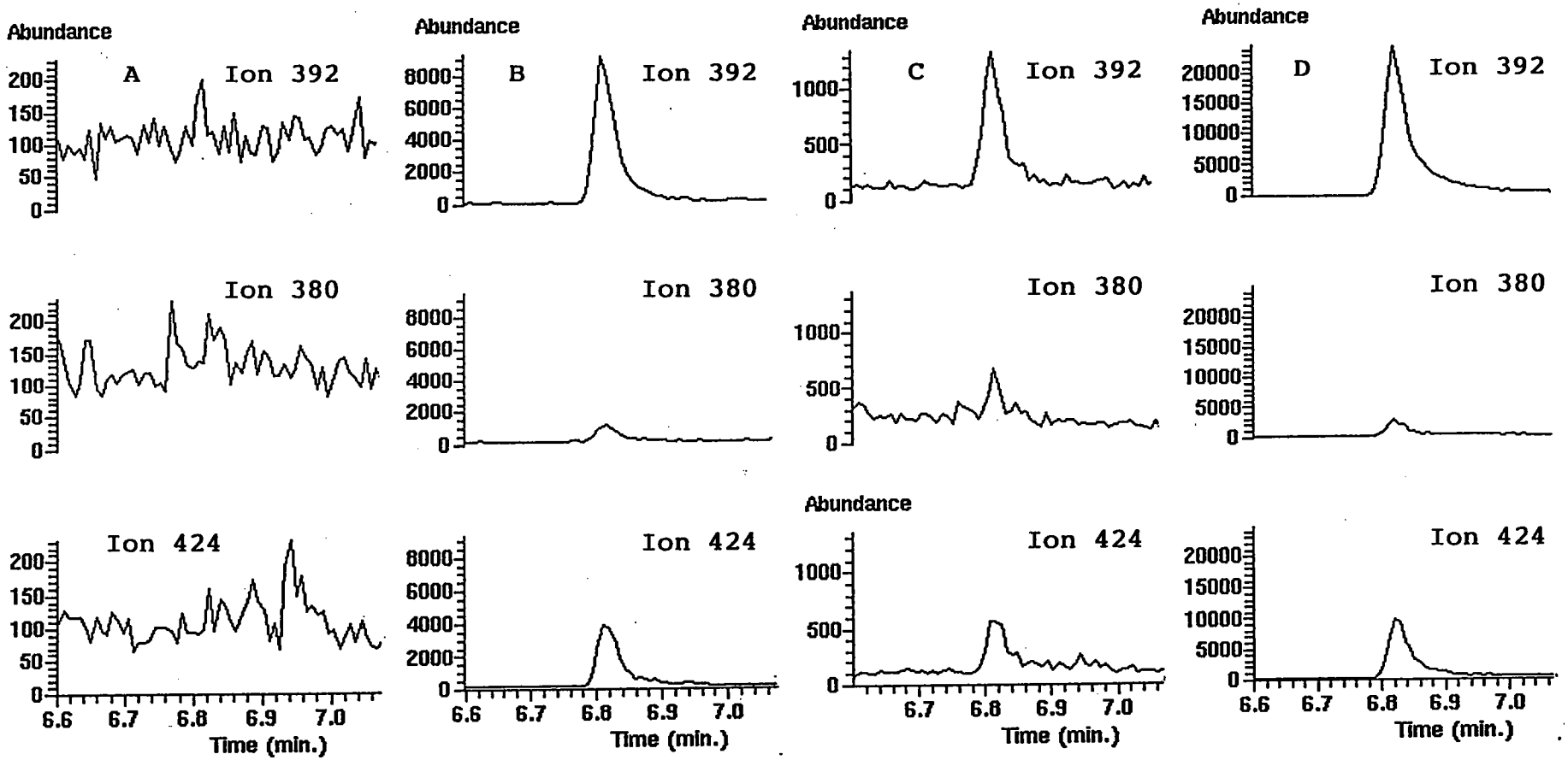

Figure 3. Selected ion traces for buprenorphine (m/z= 392, 380, 424) after the sublingual administration of 0.2mg of buprenorphine. Traces: (A) Blank; (B) T= 3.9 hours; (C) T= 19 hours and (D) urine spiked to 20ng/ml with buprenorphine

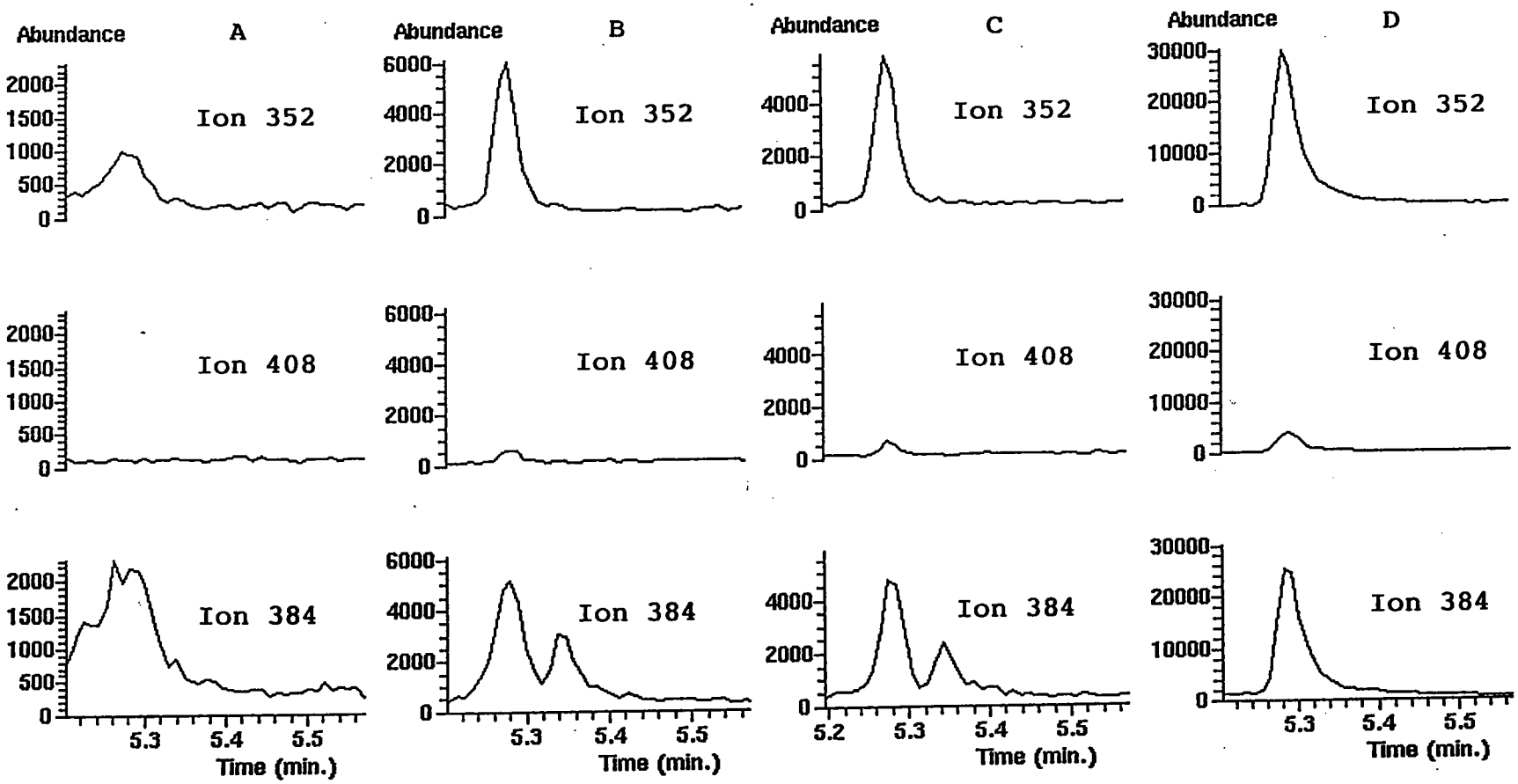

Figure 4. Selected ion traces for norbuprenorphine (m/z= 352, 408, 384) after the sublingual administration of 0.2mg of buprenorphine. Traces: (A) Blank; (B) T= 3.9 hours; (C) T= 19 hours and (D) urine spiked to 20ng/ml with norbuprenorphine