ELSEVIER

Journal of Chromatography B, 729 (1999) 341–346

JOURNAL OF CHROMATOGRAPHY B

Short communication

Detection and identification of morphine in urine extracts using thin-layer chromatography and tandem mass spectrometry

F. Tames[a], I.D. Watson[a], W. Morden[b], I.D. Wilson[c,*]

[a]*Dept. Clinical Biochemistry, University Hospital, Aintree, Lower Lane, Liverpool L9 7AL, UK*
[b]*LGC, The Heath, Runcorn, Cheshire WA7 4QD, UK*
[c]*Zeneca Pharmaceuticals, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK*

Received 28 December 1998; received in revised form 15 March 1999; accepted 16 March 1999

Abstract

The application of tandem mass spectrometry to the analysis and identification of morphine following thin-layer chromatography is described. FAB–mass spectrometry and mass spectrometry–mass spectrometry were performed following chromatography on silica gel high-performance thin-layer chromatography plates. The successful application of this simple methodology to a urine extract suggests that this approach has practical utility for confirming the identity of abused drugs detected by thin-layer chromatography. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Morphine

1. Introduction

Thin-layer chromatography remains a popular method for the screening of samples such as urine for drugs of abuse [1]. In situations where the presence of a particular compound is indicated by this type of screening it is usual for confirmation to be performed with another method (e.g. GC [2,3], GC–MS [4], HPLC [5] or HPLC–MSMS [6]). Very often this requires additional sample preparation (extraction, derivatisation etc.).

There are however, numerous examples of a range of mass spectrometric (MS) techniques used directly on the TLC spot/band for identification (reviewed in refs. [7,8]). This approach would appear to provide a rapid and efficient alternative to confirmation of the identity of TLC spots by GC–MS etc. In previous investigations we have demonstrated the utility of TLC coupled to FAB–tandem mass spectrometry (MS–MS) for the identification of natural products [9], drugs and metabolites [10–12] and industrial chemicals [13]. This work has now been extended to drugs of abuse and here preliminary results of studies on the TLC–MS and TLC–MS–MS of morphine in urine extracts are presented.

2. Materials and methods

Drug standards for morphine, codeine, methadone, dihydrocodeine and 6-monoacetylmorphine were obtained from Sigma–Aldrich (Poole, UK).

Human urine samples were collected, under direct observation, from clients attending for methadone replacement therapy at a Cheshire Community Drug Clinic. The study urine, selected on the basis of the

*Corresponding author.

0378-4347/99/$ – see front matter © 1999 Elsevier Science B.V. All rights reserved.
PII: S0378-4347(99)00120-6

screening procedure described below, was provided by a 25 year old male client who had been abusing drugs for over 4 years. He was prescribed Methadone Linctus (methadone hydrochloride 2 mg/5 ml) 55 ml/day and diazepam (20 mg/day) for his opiate addiction. The client was also using street heroin (ca. 0.5 g/day), cyclizine, cannabis and cocaine on a regular basis. On arrival at the laboratory, urine specimens were stored frozen at −20°C until analysis by the procedures detailed below. Blank urine specimens were also collected (from laboratory volunteers) into which morphine was spiked to a final concentration of 1.5 mg/l to act as a positive control. Drug standards were prepared in HPLC grade methanol (Rathburn Walkerburn, UK) at a final concentration of 1 mg/l (free drug).

2.1. Sample assay

After thawing, centrifugation at 500 *g* for 4 min and standard checks on pH, specific gravity and creatinine concentration, laboratory screening of test urine specimens for the presence of opiate drugs was by a commercially available homogenous enzyme immunoassay (EIA) technique (Diagnostic Reagents, Santa Ana, CA, USA). EIA was performed on a Kone Ultra Laboratory Analyser (LabMedics, Stockport, UK) following the manufacturers recommendations regarding sample and reagent volumes, incubation times, temperatures and cut-off concentrations to determine a positive result.

Confirmation of the positive result on this specimen was performed, using our standard in-house techniques, by reversed-phase HPLC with electrochemical detection (ED), HPTLC on silica gel with detection of morphine using the iodoplatinate reagent and capillary GC on a DB5-MS column with nitrogen–phosphorous detection.

2.2. High performance thin-layer chromatography–MS–MS

2.2.1. Sample preparation

Urine samples (1 ml) (blank, spiked blank and test) were subjected to solid-phase extraction (SPE) on SPEC.PLUS 3 ml MP1 (30 mg) columns (Thames Restek, Windsor, UK). Columns were conditioned with water (0.5 ml), 0.1 *M* acetic acid (0.5 ml) and methanol (0.5 ml) followed by drying under vacuum (18 in. Hg) for 5 min. Following sample application the analytes were eluted with ethyl acetate–methanol–concentrated aqueous ammonia (8:2:0.2, v/v, 1 ml). The eluate was reduced to dryness at 30°C under a gentle stream of dry nitrogen followed by dissolution (with vortex mixing) in 30 μl of propan-2-ol and storage at 4°C prior to HPTLC.

2.2.2. Chromatography

TLC was performed on 10×10 cm glass backed silica gel 60, 0.2 mm thick HPTLC plates containing a fluorescent indicator (E. Merck, Poole, UK). The plates were used as supplied but were activated for 1 h at 120°C and then used immediately. Aliquots (10 μl) of the extracts of the test samples were applied to the origin of the TLC plate manually using glass capillaries. Drug standards (morphine, codeine and dihydrocodeine (1 mg/ml) and 6-monoacetylmorphine (0.1 mg/ml)) were applied to the same plates for comparison of R_f etc.

In this system morphine has an R_f of 0.4, whilst the equivalent values for codeine, dihydrocodeine and 6-monoacetylmorphine were 0.28, 0.34 and 0.58 respectively. The plates were developed in an unsaturated tank (ca. 10×10×3 cm) with ethyl acetate–methanol–concentrated aqueous ammonia (8:1.3:0.4, v/v, 10 ml) for 4 cm following which the plates were air dried and then developed in ethyl acetate–methanol–concentrated aqueous ammonia (8:0.2:0.05 v/v, 10 ml) for 5 cm. The plates were then air dried for 1 h at room temperature and the spots visualised under UV light at 254 nm.

2.3. Mass spectrometry

MS and MSMS data were acquired with a VG Analytical (Fisons Instruments, Manchester, UK) ZAB–HSQ tandem mass spectrometer of BEqQ geometry. All samples were ionised by fast atom bombardment (FAB) using fast xenon atoms at 8 kV accelerating potential, and at an emission current of 1.5 mA. The ion source temperature was maintained at ambient.

MSMS data were acquired at a collision energy of 10–20 eV in the laboratory frame of reference.

Argon at an indicated pressure of 3.0×10^{-6} mbar was used as a collision gas.

All data were acquired and processed by a MSS (Mass Spectrometry Services, Manchester, UK) MASPEC Data System. The MS data were acquired in real-time centroiding mode, whereas the MS–MS were acquired in peak profile mode.

Sample preparation for TLC–MS–MS was performed as described by Oka et al. [14]. In this method the silica surrounding the spot corresponding to morphine was removed on three sides leaving a trapezoidal shaped zone. Methanol was then applied to the open end resulting in the analyte migrating towards the narrow closed end of the trapezoid where it became concentrated. A few μl of the FAB matrix (glycerol) was then applied to the top 0.5–1 mm of the zone of silica containing the concentrated analyte so as to moisten it. The treated silica gel was then carefully excised and placed on the target of the FAB probe where it was slurried with a further quantity of matrix and 2 μl of 1% trifluoroacetic acid (TFA) in methanol. Reference standards were treated in the same manner.

3. Results and discussion

Following a positive result in the preliminary immunoassay screen on the test urine confirmation of the presence of morphine was sought via our stan-

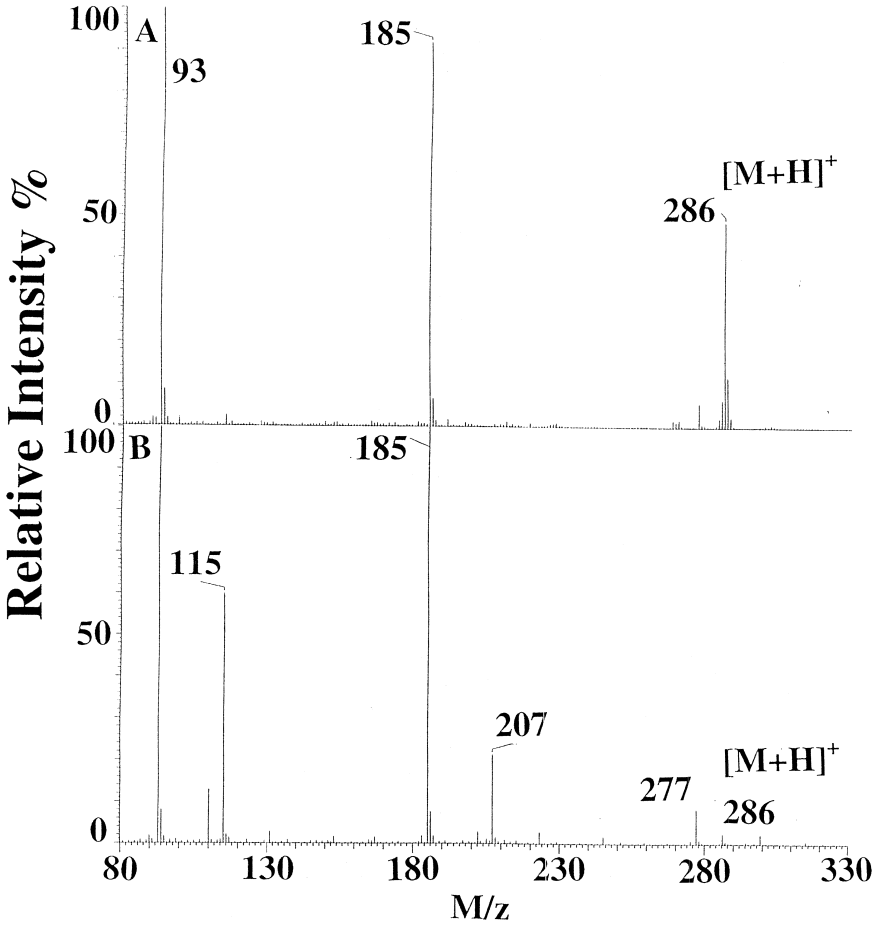

Fig. 1. The double focusing HPTLC–FABMS spectra obtained directly from the silica gel of the HPTLC plate for (A) a spot for a pure standard of morphine and (B) a spot corresponding to morphine obtained from HPTLC of a urine extract.

dard HPTLC–iodoplatinate [15], HPLC–EC [5] and GC–NPD methods [2]. With the strong support that morphine was indeed present provided by these chromatographic methods we then proceeded to investigate the use of HPTLC–MS and HPTLC–MS–MS as a means of providing unequivocal confirmation of the presence of the drug in this sample.

HPTLC–FABMS of a morphine standard gave the result shown in Fig. 1A. The $[M+H]^+$ ion for morphine was clearly visible in the FABMS spectrum at m/z 286, with ions at 93 and 185 amu due to the matrix. This spectrum was identical to that of the standard when subjected to FABMS directly from the probe, in the absence of silica gel, and corresponded to ca. 5 μg of material on the plate. This type of information used together with the R_f from the TLC separation might be adequate in confirming the identity of a pure standard or a spiked urine. However, the same treatment applied to the urine extract obtained from an addict, shown in Fig. 1b is less convincing. Whilst it is possible to detect an ion at 286 amu it was very weak compared to the intense ions due to the matrix components. This spot contained ca. 300–500 ng of morphine (by HPLC–EC and GC–NPD). Based on such a result we would suggest that unequivocal identification of morphine using HPTLC–MS would not be possible for this extract.

HPTLC–FABMS–MS on the $[M+H]^+$ ion of the morphine standard gave the complex spectrum shown in Fig. 2A, and a similar result was also obtained for the spiked urine sample. Clearly this spectrum provides a complex envelope of ions that form a useful 'fingerprint' for the analyte.

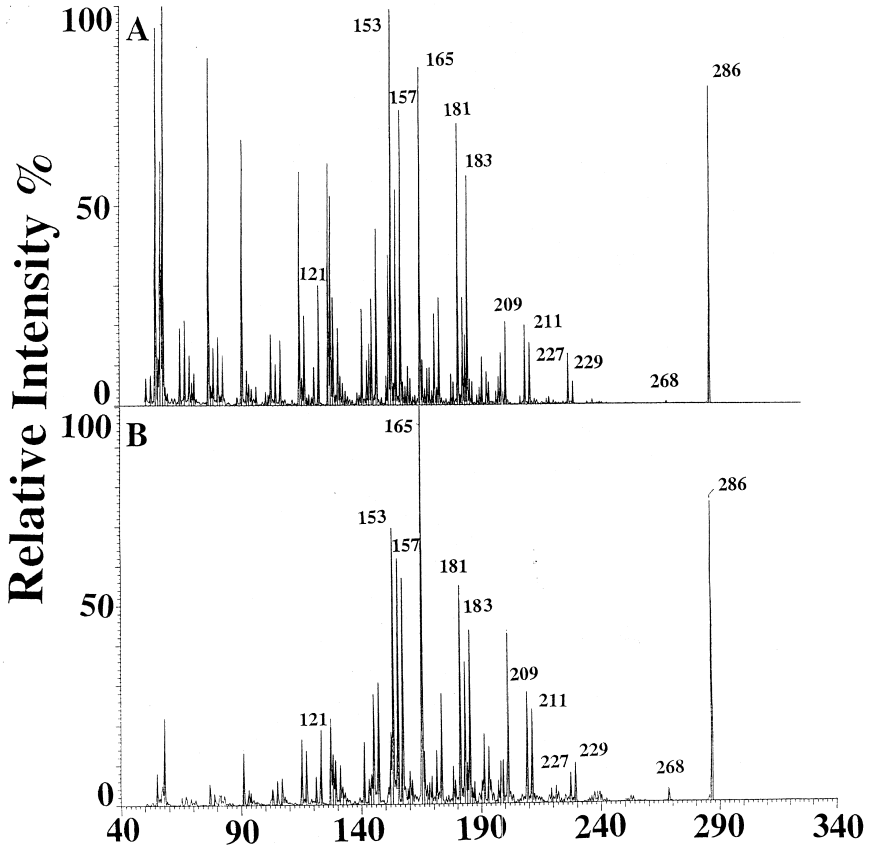

Fig. 2. The HPTLC–FABMS–MS spectra obtained from the ion at m/z 286 for (A) the spot of the pure standard of morphine shown in Fig. 1A and B the spot corresponding to morphine seen on the HPTLC of a urine extract and shown in Fig. 1B. The proposed fragmentation map is shown in Fig. 3.

The spectrum illustrated in Fig. 2B was obtained for the ion at m/z 286 present in the urine extract obtained from the addict. Whilst the relative intensities of the various ions are not identical to that of the standard the overall 'fingerprint' is qualitatively the same, and would provide the basis for a confident

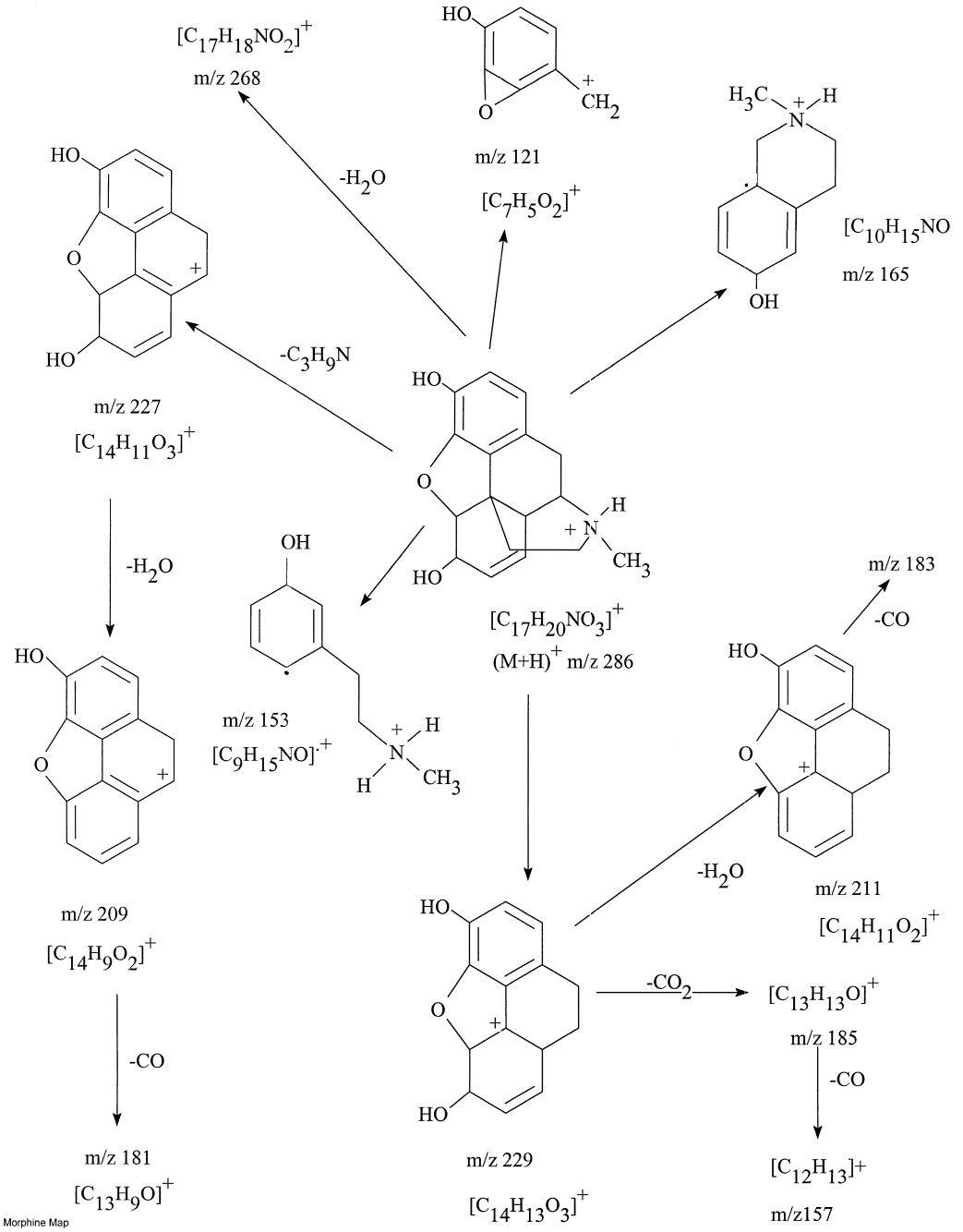

Fig. 3. A proposed fragmentation map for the ions observed for morphine under the TLC–MS–MS conditions describe here.

identification. A proposed fragmentation map for the major ions in the product ion spectrum are shown in Fig. 3.

The whole HPTLC–MS–MS procedure can be performed relatively rapidly, and it is possible to obtain spectral data within 5–10 min of beginning to process the developed TLC plate. By comparison the time taken for GC–NPD (extraction, derivatisation and chromatography), performed after the HPTLC screening of the urine samples, amounts to several hours.

4. Conclusions

We have previously shown the potential for HPTLC–MS–MS to identify drugs and metabolites in human urine extracts with successful applications to antipyrine [10] and non-steroidal anti-inflammatories [12]. This study represents the first application of HPTLC–MS–MS without substance elution to the detection and identification of a drug of abuse in urine and highlights the potential of the technique in this area. HPTLC is already used extensively for screening urine samples for drugs of abuse and the extension of this to include HPTLC–MS–MS may thus provide the basis for a rapid and technically undemanding means of confirmation of identity for such compounds. The use of HPTLC–MS might be suitable for relatively pure substances, where relatively large amounts of material could be loaded onto the plate however it would probably be inadequate for urine extracts. The extra spectral information provided by MS–MS is thus essential in providing the confidence that it is morphine and not some co-chromatographing interferent that is present in the sample. Further investigations are in progress to determine the suitability of HPTLC–MS–MS for other classes of drugs of abuse such as benzodiazepines.

Acknowledgements

The expert advice of Dr. E. Clayton is gratefully acknowledged.

References

[1] A.C. Moffat, J.-P. Franke, A.H. Stead, A.H.R. Gill, B.S. Finkle, M.R. Moller, R.K. Muller, F. Wunsch, R.A. de Zeeuw, Thin-layer chromatographic R_f values of toxicologically relavent substances on standardized systems, VCH Verlagsgesellschaft, Weinheim, 1987.
[2] R. Caldwell, H. Challenger, Ann. Clin. Biochem. 26 (1989) 430.
[3] F.T. Delbeke, M. Debackere, J. Pharm. Biomed. Anal. 9 (1991) 959.
[4] W.H. Phillips Jr., K. Ota, N.A. Wade, J. Anal. Tox. 13 (1989) 269.
[5] B. Law, C.P. Goddard, M. Japp, I.S. Humphreys, J. Forensic Sci. 24 (1983) 561.
[6] M. Zheng, K.M. McErlane, M.C. Ong, J. Pharm. Biomed. Anal. 16 (1998) 971.
[7] G.W. Somsen, W. Morden, I.D. Wilson, J. Chromatogr. 703 (1995) 613.
[8] K.L. Busch, Handbook of Thin-layer Chromatography, in: J. Sherma, B. Fried (Eds.), 2nd ed, Marcel Dekker, New York, 1996, pp. 241–272.
[9] I.D. Wilson, R. Lafont, R.G. Kingston, C.J. Porter, J. Planar Chromatogr. 3 (1990) 359.
[10] P. Martin, W. Morden, P. Wall, I.D. Wilson, J. Planar Chromatogr. 5 (1992) 255.
[11] I.D. Wilson, W. Morden, J. Planar Chromatogr. 4 (1991) 226.
[12] W. Morden, I.D. Wilson, Rapid Comm. Mass Spectrom. 10 (1996) 1951.
[13] J.J. Monaghan, W.E. Morden, T. Johnson, I.D. Wilson, P. Martin, Rapid Commun. Mass Spectrom. 6 (1992) 608.
[14] H. Oka, Y. Ikai, N. Kawamura, J. Hayakawa, K. Masuda, K.-I. Harada, M. Suzuki, Rapid Commun. Mass Spectrom. 6 (1992) 89.
[15] J. Blakesley, D. Wood, C. Hiwse, J. Spencer-Peet, Ann. Clin. Biochem. 24 (1987) 508.