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Journal of Chromatography B, 685 (1996) 67–80

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Solid-phase extraction and derivatisation methods for β-blockers in human post mortem whole blood, urine and equine urine

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Received 14 August 1995; revised 28 February 1996; accepted 11 March 1996

Abstract

This paper details various rapid and sensitive methods for the extraction and derivatisation of propranolol, metoprolol, sotalol, atenolol, pindolol, timolol, oxprenolol, alprenolol and penbutolol in equine urine and in human post mortem whole blood and urine. Three solid-phase extraction methods are described involving the use of either XtrackT XRDAH515, Bond Elut Certify or Sep-Pak C_{18} cartridges. Two derivatisation methods are also described involving the formation of cyclised silyl or pentafluoropropionate derivatives with either chloromethyldimethylchlorosilane or pentafluoropropionic anhydride, respectively. Gas chromatographic–mass spectrometry analysis was carried out in select-ion monitoring mode. All these methods were evaluated using drug-free human post mortem blood, urine and equine urine fortified at various levels with the β-blockers mentioned above. The application of some of these methods on a forensic case study is also presented. This work does not include samples from equine administration trials of β-blockers.

Keywords: β-Blockers; Propanolol; Metoprolol; Sotalol; Atenolol; Pindolol; Timolol; Oxprenolol; Alprenolol; Penbutolol

1. Introduction

Propranolol, metoprolol, sotalol, timolol, oxprenolol, alprenolol, atenolol, pindolol and penbutolol are β-adrenoceptor blocking drugs used for the treatment of various cardiovascular disorders such as hypertension, angina pectoris and cardiac arrhythmia. All of the above drugs are prohibited substances under the rules of racing. There are many references in the literature for the analysis of these β-blockers in serum, urine and tissues [1–25] but very few papers refer to the analysis of whole blood [26]. Forensic toxicological analysis of drugs is generally performed on whole blood and equine urine presents peculiar extraction problems. Methods have been developed for the analysis of β-blockers in both these media.

It is difficult to analyse these nine β-blockers simultaneously using liquid–liquid extraction. Their relatively low solubilities in organic solvents and their different pK_a values [27] lead to different levels of partitioning into organic solvents. The solid-phase extraction methods presented here are based on the manipulation of hydrophobic–hydrophilic and ionic interactions, making simultaneous analysis of all nine β-blockers possible.

Various single and dual column solid-phase extraction methods suitable for different sample matrices were evaluated using drug-free human post-mortem blood, urine and equine urine fortified at various levels with the above mentioned β-blockers.

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0378-4347/96/$15.00
PII S0378-4347(96)00140-5

These solid-phase extraction methods use XtrackT XRDAH515, Bond Elut Certify and Sep-Pak C_{18} cartridges. Cyclised silyl, pentafluoropropionate (PFP), trimethylsilyl-trifluoroacetyl (TMS-TFA) and cyclic boronate derivatives of nine β-blockers were compared.

The application of some of these methods on a forensic case study is also presented.

Although cyclised silyl derivatives were first described by Hammar [1] and Hoffman et al. [2], the method of forming cyclised silyl derivatives of β-blockers in our laboratory was adopted from Dumasia and Houghton [3]. PFP derivatives of some β-blockers have been described by Wan et al. [4] for gas chromatography (GC)–electron-capture detection analysis and later by Ciardi et al. [5] for GC–NICI analysis.

2. Experimental

2.1. Reagents

All reagents used were analytical grade. All solvents were distilled. Only distilled or deionised water was used.

All β-blockers were supplied by their manufacturers: propranolol hydrochloride, oxprenolol hydrochloride (Fisons, Thornleigh, Australia); metoprolol tartrate, sotalol hydrochloride, alprenolol hydrochloride (Astra, North Ryde, Australia); atenolol, bamethane sulphate (Sigma, St. Louis, MO, USA); penbutolol sulphate (Hoechst, Frankfurt, Germany); timolol maleate (Merk Sharp and Dohme, Granville, Australia); pindolol base (Sandoz Australia, North Ryde, Australia).

For acetate–acetic acid buffer, a saturated solution of sodium acetate in 500 ml water was prepared and the pH was adjusted to 5.0±0.2 with glacial acetic acid. Methanol and acetonitrile were high-performance liquid chromatography grade.

For β-glucuronidase with sulphatase activity, the contents of a bottle of β-glucuronidase (Patella vulgata 250 000 units, supplied by Sigma) were dissolved in 20 ml water. A 1-g amount of sodium acetate was added and the pH was adjusted to 5.0±0.2 with acetate–acetic buffer.

For 0.1 *M* phosphate buffer (pH 6.0), 3.4 g of KH_2PO_4 was dissolved in 250 ml water and the pH was adjusted to 6.0±0.2 with 2 *M* NaOH. For 0.01 *M* phosphate buffer (pH 7.0), 0.34 g of KH_2PO_4 was dissolved in 250 ml water and the pH was adjusted to 7.0±0.2 with 2 *M* NaOH. A 1 *M* acetic acid solution was prepared by dissolving 5.75 ml glacial acetic acid in 100 ml water.

Bond Elut Certify cartridges (500 mg bed) were supplied by Varian (Harbor City, CA, USA). Sep-Pak C_{18} cartridges (500 mg bed) were supplied by Waters (Milford, MA, USA). XtrackT XRDAH515 cartridges (500 mg bed) were supplied by Worldwide Monitoring (Horsham, PA, USA).

Chloromethyldimethylchlorosilane (CMDMCS), pentafluoropropionic anhydride (PFPA), N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) and N-methylbistrifluoroacetamide (MBTFA) were supplied by Alltech Associates (Homebush, Australia).

For the CMDMCS derivatising mixture, 2 ml hexane was combined with 150 μl CMDMCS and 150 μl diethylamine. The mixture was vortex mixed and centrifuged at approximately 1000 *g* (2500 rpm) for 5 min. The supernatant was used for derivatisation.

Methylboronic acid (MBA) was supplied by Aldrich (Milwaukee, WI, USA). For MBA derivatising reagent, 0.125 g MBA were dissolved in 25 ml ethyl acetate. Pyridine was distilled off potassium hydroxide (KOH) and kept over KOH. Borax solution (0.1 *M*) was prepared by dissolving 19.1 g sodium borate in 500 ml water. Na_2SO_4 (AR grade) was washed with hexane and dried at 110°C.

2.2. Gas chromatography–mass spectrometry (GC–MS)

GC–MS analyses were performed using a Hewlett-Packard 5890 GC attached to a 5971 MS detector. A fused-silica capillary column DB-1 was used (25m×0.2mm I.D., 0.25 μm film thickness, Alltech Associates). The injection port was in splitless mode.

Analyses were performed at a helium flow-rate of 0.6 ml/min and the temperature settings were: injection temperature 260°C; initial column temperature 140°C; initial hold time 2.5 min; temperature rate 20°C/min; final column temperature 300°C; final

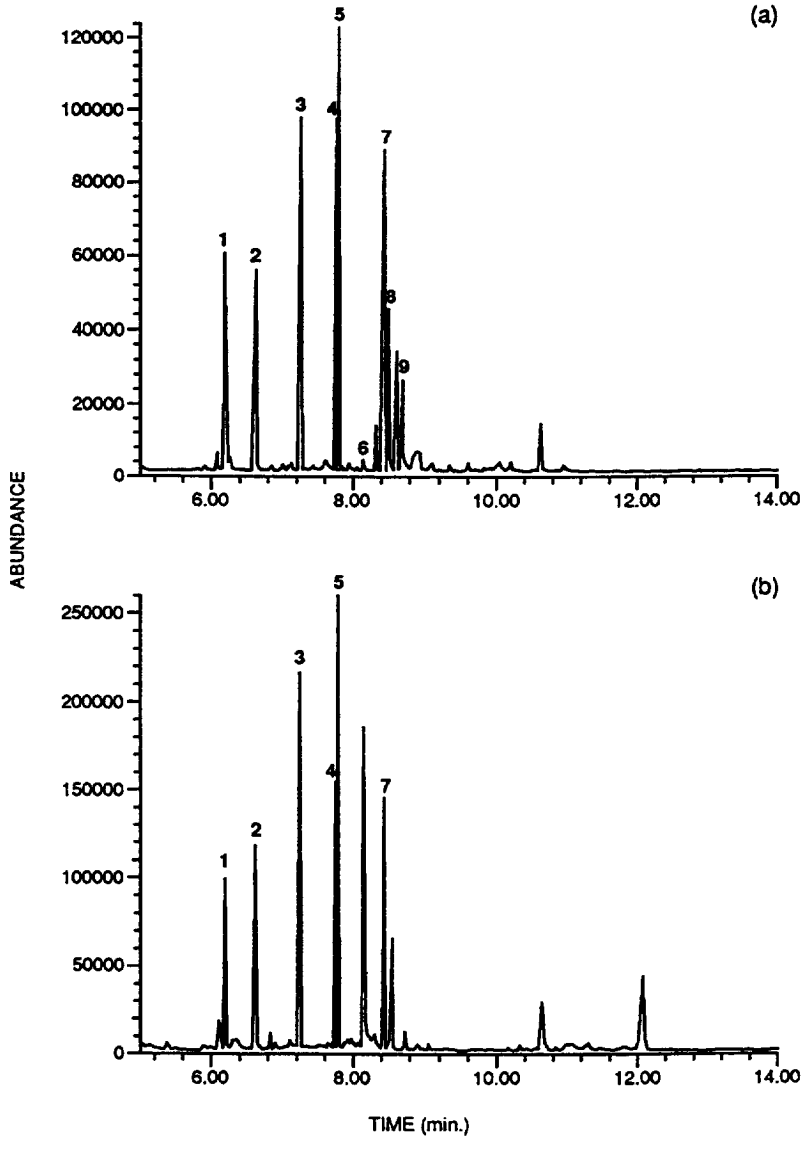

Fig. 1. Reconstructed TIC from GC–SIM-MS analysis of cyclised silyl derivatives of (1) alprenolol, (2) oxprenolol, (3) metoprolol, (4) propranolol, (5) penbutolol and bamethane, (6) pindolol, (7) timolol, (8) sotalol and (9) atenolol extracted from spiked post mortem blood using (a) Bond Elut Certify and (b) XtrackT XRDAH515 cartridges. The samples contained 500 ng/ml of each β-blocker.

hold time 5 min; transfer line temperature 290°C and ion source temperature 290°C. The mass spectra were taken in the electron impact (EI) mode at 70 eV. The EI mass spectra of the cyclised silyl, TMS-TFA and cyclic boronate derivatives of β-blockers were obtained in full scan mode in the mass range 40–500 a.m.u. and in the range of 50–750 a.m.u. for PFP derivatives. Analyses of blood and urine extracts were performed in the selected ion monitoring (SIM) mode by monitoring specific ions of the cyclised

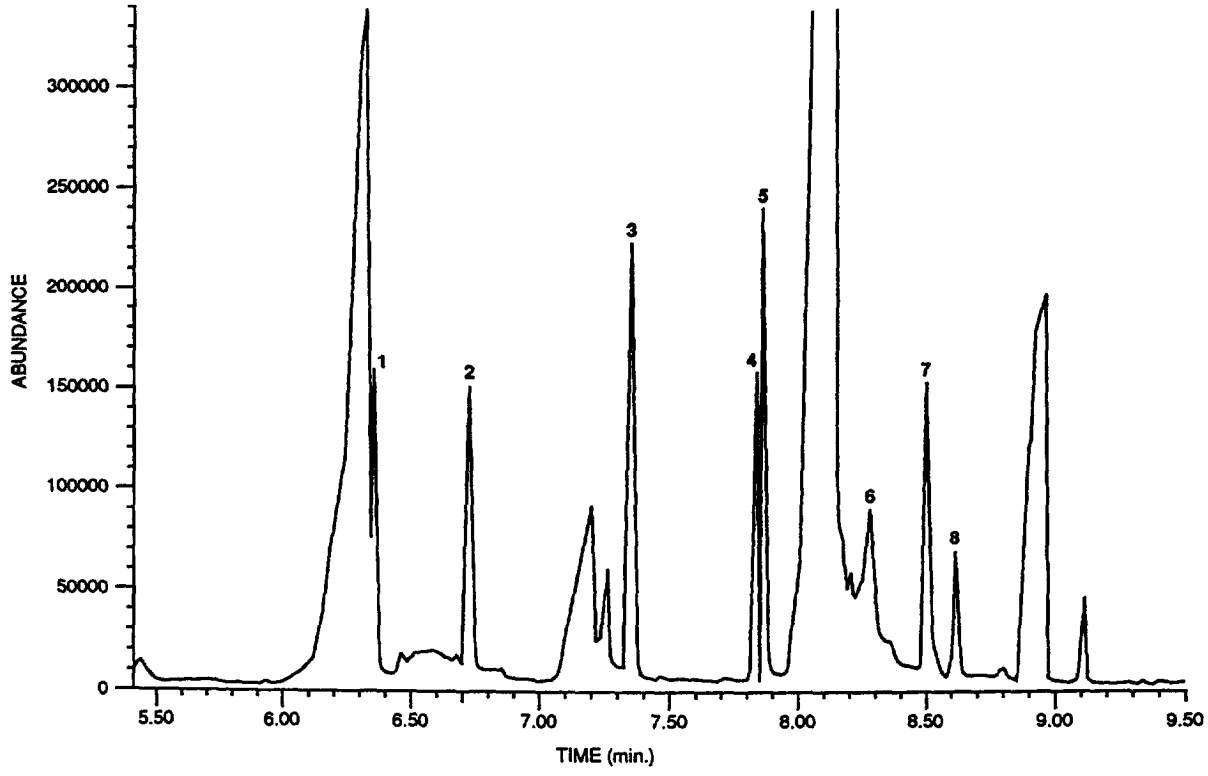

Fig. 2. Reconstructed TIC from GC–SIM-MS analysis of cyclised silyl derivatives of (1) alprenolol, (2) oxprenolol, (3) metoprolol, (4) propranolol, (5) penbutolol and bamethane, (6) pindolol, (7) timolol and (8) sotalol extracted from spiked post mortem blood using Sep-Pak C_{18} cartridge. The sample contained 500 ng/ml of each β-blocker.

silyl derivatives and these ions are underlined in Table 2 (see below).

2.3. Sample preparation

The matrices used in this study consisted of drug-free human post mortem blood, urine and equine urine fortified at various levels with β-blockers.

2.3.1. Post mortem blood and urine samples for analysis of unconjugated β-blockers

To facilitate the passage of whole blood through a solid-phase extraction cartridge, it was necessary to dilute, ultrasonicate (to cause lysis of blood cells) and centrifuge the blood.

Blood (1 g) or urine (1 g) was combined with 50 μl of internal standard solution (10 μg/ml bamethane in methanol) and 10 ml 0.1 *M* phosphate buffer. The mixture was vortex mixed and the pH was adjusted to 6.0±0.2 with 2 *M* NaOH or 2 *M* H_3PO_4 for methods A and B. For method C, the pH was adjusted to 7.0±0.2 with 2 *M* NaOH or 2 *M* H_3PO_4. The mixture was left in an ultrasonic bath for 10 min and centrifuged at approximately 1000 *g* (2500 rpm) for 10 min.

2.3.2. Equine urine samples for analysis of conjugated and unconjugated β-blockers

Urine (5 ml) was combined with 1 ml acetate–acetic acid buffer and the pH was adjusted to 5.0±0.2. β-Glucuronidase (100 μl, equivalent to

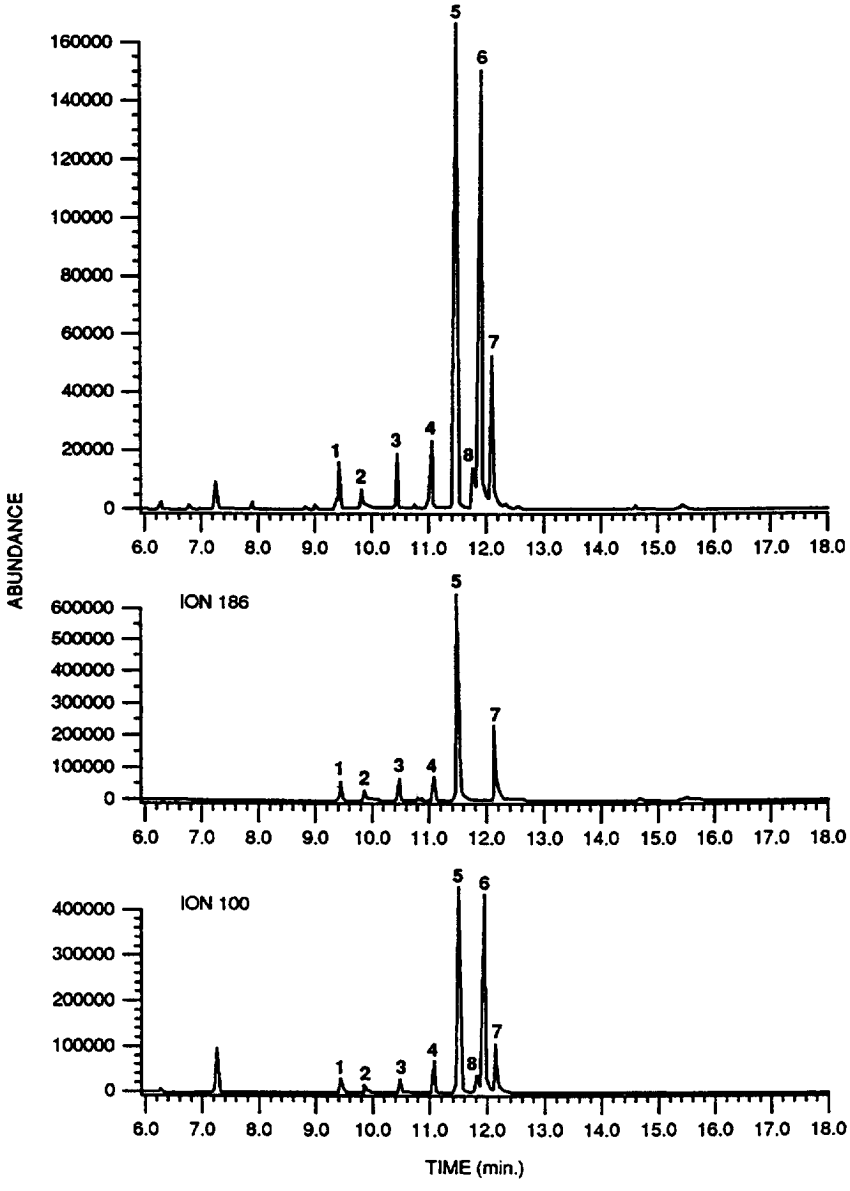

Fig. 3. Reconstructed TIC from GC–SIM-MS analysis and selected ion chromatograms of cyclised silyl derivatives of β-blockers. These were extracted from spiked equine urine using combined Sep-Pak C_{18} and XtrackT XRDAH515 cartridges. The sample contained 2.5 μg/ml of each of (1) alprenolol, (2) oxprenolol, (3) metoprolol, (4) propranolol and penbutolol (coeluting), (5) 130 μg/ml pindolol, (6) 500 μg/ml sotalol, (7) 130 μg/ml atenolol and (8) 2.5 μg/ml timolol.

1250 units of activity) was added and the mixture was left overnight in a water bath at 37°C. Phosphate buffer (0.01 *M*, 500 μl, pH 7.0) was added and the pH was adjusted to 7.0±0.2 with 2 *M* NaOH or 2 *M* H_3PO_4. The mixture was centrifuged at approximately 1000 *g* (2500 rpm) for 10 min.

2.4. Extraction procedure for human whole blood and urine

2.4.1. Method A: extraction using XtrackT XRDAH515 cartridge

The cartridge was pre-conditioned by passing through 5 ml methanol, 5 ml water and 2 ml 0.1 *M* phosphate buffer under full vacuum. The cartridge was prevented from running dry. The prepared blood or urine sample was passed through at low vacuum (ca. $1.7 \cdot 10^4$ Pa). The cartridge was washed with 5 ml water, 5 ml methanol and allowed to dry under full vacuum for 5 min. The β-blocker was eluted by passing 5 ml 4% (v/v) conc. ammonia in methanol at low vacuum. The extract was evaporated to dryness in a water bath under a stream of air.

2.4.2. Method B: extraction using Bond Elut Certify cartridge

The cartridge was pre-conditioned with 2 ml methanol followed by 2 ml 0.1 *M* phosphate buffer under full vacuum. The cartridge was prevented from running dry. The prepared blood or urine sample was passed through at low vacuum (ca. $1.7 \cdot 10^4$ Pa). The cartridge was washed with 1 ml 1 *M* acetic acid and allowed to dry under full vacuum for 5 min. Methanol (6 ml) was passed through the cartridge, which was then allowed to dry under full vacuum for 2 min. The β-blocker was eluted by passing 3×2 ml 4% (v/v) concentrated ammonia in ethyl acetate at low vacuum. The extract was evaporated to dryness in a water bath under a stream of air.

2.4.3. Method C: extraction using Sep-Pak C_{18} cartridge

The cartridge was pre-conditioned by passing through 3 ml methanol, 6 ml 4%(v/v) conc. ammonia in methanol, 3 ml acetonitrile and 6 ml water under full vacuum. The cartridge was prevented from running dry. The prepared blood or urine sample was passed through the cartridge at low vacuum (ca. $1.7 \cdot 10^4$ Pa). The cartridge was then washed with 9 ml water, 9 ml hexane and allowed to dry under full vacuum for 2 min. The β-blocker was eluted by passing 3 ml of 4% (v/v) concentrated ammonia in methanol at low vacuum. The eluate was evaporated to dryness in a water bath under a stream of air.

2.5. Extraction procedure for equine urine

2.5.1. Method D: dual column solid-phase extraction using Sep-Pak C_{18} and XtrackT XRDAH515 cartridges

The prepared urine sample was passed through a pre-conditioned Sep-Pak C_{18} cartridge as described in method C. The dry extract was dissolved in 3 ml 0.1 *M* phosphate buffer and ultrasonicated for 10 min. This sample was then passed through a pre-conditioned XtrackT XRDAH515 cartridge as described in method A.

2.6. Derivatisation of β-blockers

2.6.1. Formation of cyclised silyl derivative

The dry residue was vortex mixed with 25 μl toluene and 150 μl CMDMCS derivatising mixture was added. The mixture was vortex mixed and then heated at 80°C for 20 min. The mixture was evaporated to dryness under N_2, reconstituted in 200 μl toluene and an aliquot of 1 μl was injected onto the GC–MS system.

2.6.2. Formation of PFP derivative

The dry residue was combined with 500 μl cyclohexane, 50 μl pyridine and 100 μl PFPA. The mixture was vortex mixed and then heated at 80°C for 30 min. Cyclohexane (3 ml) and 1 ml 0.1 *M* borax solution were added and the mixture was

Table 1
Estimated lower limit of detection of cyclised silyl derivatives of β-blockers after extraction of spiked human blood or spiked urine using either solid-phase extraction methods A, B or C

β-Blocker	Detection limit (mg/l)
Alprenolol	0.01
Oxprenolol	0.01
Metoprolol	0.01
Propranolol	0.01
Penbutolol	0.01
Pindolol	0.05
Sotalol	0.1
Timolol	0.03
Atenolol	0.2

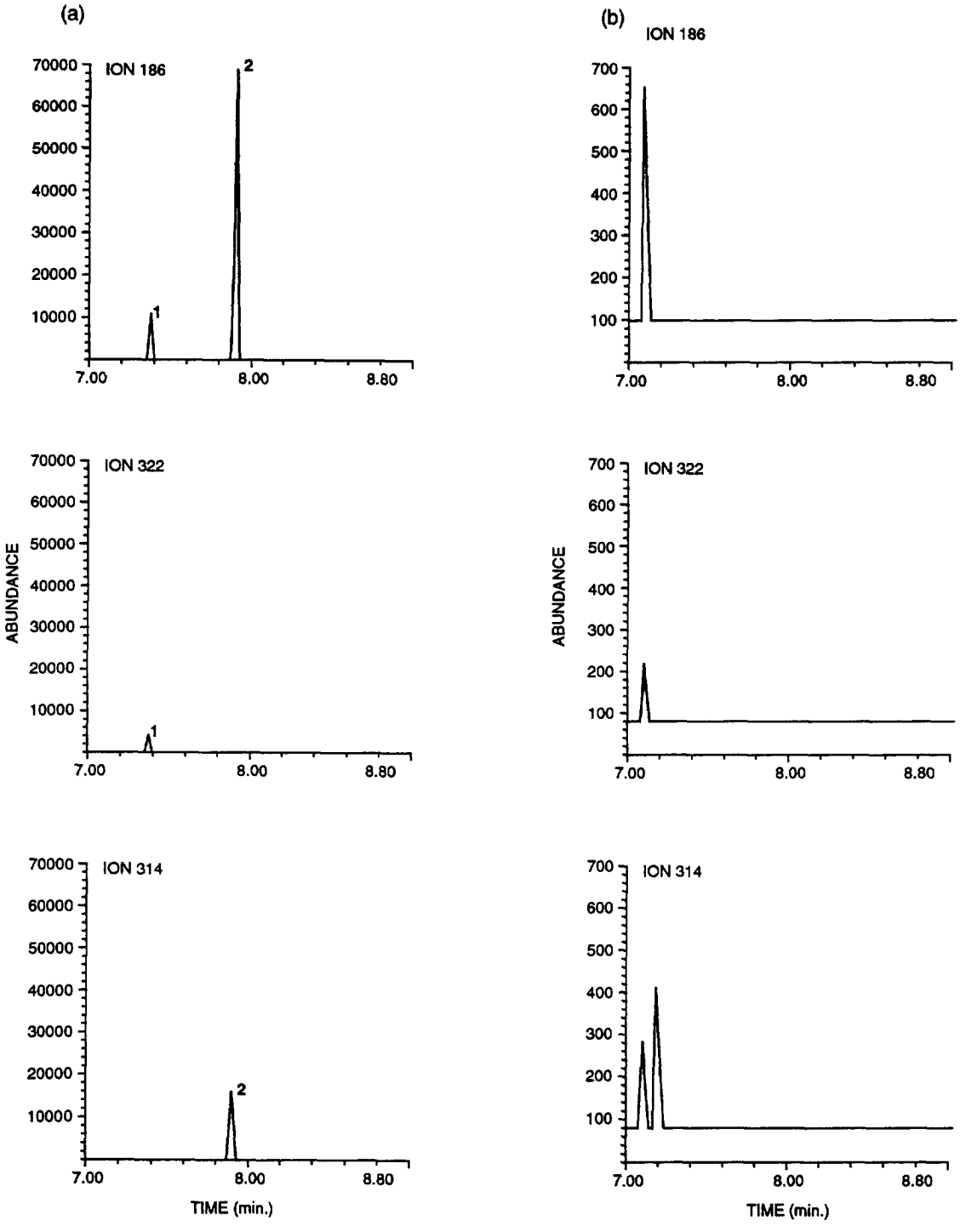

Fig. 4. Selected ion chromatograms of (a) a forensic case post mortem blood sample containing (1) metoprolol and (2) propranolol as internal standard and (b) drug-free post mortem blood used to prepare the calibration standards.

vortex mixed. The organic layer was dried with Na_2SO_4, evaporated to dryness under N_2 and reconstituted in 200 μl hexane. An aliquot of 1 μl was injected onto the GC–MS system.

The β-blockers were fully derivatised to the tris-PFP-derivative of pindolol, sotalol, atenolol and to the bis-PFP-derivative of alprenolol, oxprenolol, metoprolol, propranolol and timolol.

Table 2
Main fragment ions for cyclised silyl derivatives of β-blockers. The underlined ions are suggested for screening of β-blockers by SIM

β-Blocker	RRT[a]	Molecular mass	*m/z* (% intensity)
Alprenolol	0.856	319	186(100), 100(55), 304(85), 276(30), 59(25), 319(25)
Oxprenolol	0.895	335	186(100), 100(55), 320(50), 335(10), 294(20), 321(10)
Metoprolol	0.953	337	186(100), 100(55), 322(75), 294(30), 45(30), 337(20)
Propranolol	1.005	329	186(100), 100(55), 314(40), 115(15), 286(15), 59(20), 329(25)
Penbutolol	1.005	361	346(100), 100(25), 304(20), 144(15), 361(15), 200(10)
Pindolol	1.045	318	186(100), 100(70), 303(40), 318(30), 59(20), 275(15)
Sotalol	1.081	342	100(60), 327(100), 263(70), 299(70), 73(40), 342(20), 114(20)
Timolol	1.073	386	100(100), 144(85), 200(65), 371(65), 57(30), 386(10), 329(10)
Atenolol	1.100	336	186(100), 100(45), 321(50), 293(20), 59(20), 336(15)
Bamethane	1.000	385	342(100), 100(35), 213(40), 328(20), 385(10), 146(20), 299(10)

[a] RRT = relative t_R to bamethane.

2.6.3. Formation of TMS-TFA derivative

The derivative was prepared as described by Leloux et al. [6]. The dry residue was vortex mixed with 100 μl MSTFA and heated at 60°C for 5 min. MBTFA (30 μl) was added and the mixture was heated at 60°C for 5 min. An aliquot of 1 μl was injected onto the GC–MS system.

2.6.4. Formation of cyclic methylboronate derivative

The derivative was prepared as described by Zamecnik [7]. The dry residue was vortex mixed with 50 μl ethyl acetate and 25 μl MBA derivatising reagent was added. The mixture was vortex mixed and then heated at 80°C for 20 min. An aliquot of 1 μl was injected onto the GC–MS system.

Table 3
Main fragment ions for PFP derivatives of β-blockers. The underlined ions are suggested for screening of β-blockers by SIM

β-Blocker derivative	RRT[a]	Molecular mass	*m/z* (% intensity)
Alprenolol bis-PFP	0.849	541	202(100), 366(100), 176(70), 119(65), 131(40), 408(35), 91(30)
Oxprenolol bis-PFP	0.888	557	202(100), 366(100), 176(70), 119(40), 408(30), 147(20), 316(10)
Metoprolol bis-PFP	0.953	559	366(100), 202(75), 176(50), 408(40), 107(30), 316(30), 246(10)
Propranolol bis-PFP	1.000	551	366(100), 202(80), 115(80), 176(60), 408(30), 144(30), 246(10)
Pindolol tris-PFP	0.990	686	366(100), 202(50), 176(30),408(30), 119(30), 203(20), 278(15)
Sotalol tris-PFP	0.948	710	176(100), 218(65), 119(20), 79(10), 262(5), 289(3)
Timolol bis-PFP	1.066	608	187(100), 154(35), 57(20), 202(15), 366(17), 119(10), 172(10)
Atenolol tris-PFP	0.987	704	366(100), 202(70), 176(60), 119(35), 408(30), 132(20), 89(5)

[a] RRT = relative t_R to propranolol.

3. Results and discussion

Results shown in Fig. 1, Fig. 2 and Fig. 3 were obtained from analysis by GC–SIM-MS monitoring all ions underlined in Table 2 (see below).

Extraction of spiked human whole blood and urine using XtrackT XRDAH515 or Bond Elut Certify cartridges gave very clean extracts (Fig. 1). No interferences were found in drug-free human post mortem blood and urine samples extracted with Bond Elut Certify or XtrackT XRDAH515 cartridges and derivatised with CMDMCS. In the case of partial coelution of propranolol and penbutolol, target analysis is possible by SIM of unique ions for each drug, i.e. ions 314 and 346 respectively. Extraction with Sep-Pak C_{18} cartridges gave clean extracts from spiked human urine but the extracts from blood generally produced unacceptable chromatograms (Fig. 2).

For forensic purposes, the simultaneous screening for the nine β-blockers (propranolol, metoprolol, sotalol, atenolol, pindolol, timolol, oxprenolol, alprenolol and penbutolol) in human blood is better carried out using either XtrackT XRDAH515 or Bond Elut Certify cartridges. However, Sep-Pak C_{18} cartridges may be suitable for the screening of a specific β-blocker for which matrix interference does not occur. In the case of simultaneous screening of β-blockers in human urine, all three cartridges were suitable.

Extraction of spiked equine urine using Bond Elut Certify, XtrackT XRDAH515 or Sep-Pak C_{18} car-

Table 4
Main fragment ions for TMS-TFA derivatives of β-blockers. The underlined ions are suggested for screening of β-blockers by SIM

β-Blocker derivative	RRT[a]	Molecular mass	*m/z* (% intensity)
Alprenolol mono-TMS-TFA	0.860	417	73(100), 284(100), 129(50), 159(20), 101(20), 59(10), 228(15), 242(10), 402(1)
Oxprenolol mono-TMS-TFA	0.895	433	284(100), 73(90), 129(70), 43(40), 166(20), 242(12), 151(10), 270(3), 418(1)
Metoprolol mono-TMS-TFA	0.958	435	284(100), 73(70), 129(45), 45(30), 235(20), 101(20), 228(10), 154(10), 435(1)
Propranolol mono-TMS-TFA	1.000	427	129(100), 284(100), 73(80), 43(45), 115(40), 169(20), 101(20), 242(15), 427(3)
Pindolol mono-TMS, bis-TFA	1.084	512	73(100), 284(70), 129(60), 205(15), 242(10), 488(5), 168(5), 318(3)
Sotalol mono-TMS, bis-TFA	1.034	536	344(100), 73(50), 126(10), 250(10), 137(5), 45(5), 206(4), 272(3)
Timolol mono-TMS-TFA	1.064	484	242(100), 57(60), 129(60), 73(50), 187(30), 154(10), 101(10), 413(1)
Atenolol mono-TMS-TFA	1.088	433	73(100), 284(70), 43(30), 129(25), 101(10), 270(10), 242(10), 326(7)

[a] RRT = relative t_R to propranolol.

tridges produced dirty extracts with the latter being the worst. Although Bond Elut Certify cartridges gave the cleanest extracts out of the three columns, most equine urine samples were difficult to pass through the cartridge even when positive pressure was applied. Equine urine is a difficult medium to analyse, being a complex matrix often with the consistency of honey due to mucous material secreted by the animal. Analysis is also difficult because the matrix produces dirty extracts and thus producing high chromatography background which affects the lower detection limit of drugs. This difficulty was overcome by utilising the combination of hydrophobic–hydrophilic and ionic interactions of solid-phase extraction techniques. The combined use of Sep-Pak C_{18} and XtrackT XRDAH515 cartridges (method D) produced much cleaner extracts (Fig. 3) than when using single column extraction. The clean equine urine extracts produced resulted in the detection of β-blockers down to 100 ng/ml of urine. However lower detection limits in equine urine were not established.

The lower detection limits obtained for cyclised silyl derivatives of β-blockers after extraction from spiked human blood and spiked urine by three different solid-phase extraction methods compared favourably (Table 1). These detection limits were estimated by spiking blood and urine samples and were based on a signal to noise ratio of 4:1. Additional information on detection limits for metoprolol and propranolol was obtained from forensic case work. The estimated limits of detection in human blood fell either below or within average blood levels obtained after therapeutic oral doses of the respective β-blockers [27,28]. The method involving solid-phase extraction with Bond Elut Certify cartridges and derivatisation with CMDMCS was applied to real forensic samples (Fig. 4). The forensic case required the confirmation and quantitation of metoprolol in blood. Good sensitivity was obtained for both the screening ion for metoprolol, ion 186, and its confirmation ion of 332. Similarly propranolol chosen in this case as the internal standard showed good sensitivity for the 186 ion and specificity for the 314 ion. The drug-free post mortem blood used for the spiked calibration standards showed low abundance for observed peaks for all three ions (186, 322, 314). The peaks also were resolved from the retention times of both metoprolol and propranolol.

Table 2 and Table 3 show EI mass spectra data for cyclised silyl and PFP derivatives of β-blockers. The underlined ions are suggested for the screening of β-blockers using SIM.

Two other derivatives of β-blockers, TMS-TFA and cyclic boronates, have been discussed extensively by Leloux et al. [6] and Zamecnik [7]. The TMS-TFA derivatives have been found to be suitable for screening by SIM (Table 4). Limited work done in our laboratory on cyclic methylboronate derivatives (Table 5) indicated that the specific high mass ions generated with the cyclic boronate derivatives can be used for screening and confirmation of individual β-blockers using SIM.

Cyclised silyl derivatives of β-blockers are preferred over PFP, TMS-TFA or cyclic boronate derivatives due to their greater specificity and sensitivity. Some examples of the total ion chromatograms (TIC) for each derivative are shown in Fig. 5 and Fig. 6. Higher sensitivity is due to a minimum of

Table 5
Main fragment ions for cyclic methylboronate derivatives of β-blockers

β-Blocker derivative	RRT[a]	Molecular mass	*m/z* (% intensity)
Oxprenolol	0.857	289	274(100), 218(85), 289(20), 210(85), 124(45), 98(40)
Metoprolol	0.932	291	276(100), 291(20), 275(35), 140(20), 124(17), 98(15)
Propranolol	1.000	283	283(85), 268(50), 140(60), 124(100), 128(100), 98(75)
Pindolol	1.055	272	272(100), 257(10), 271(35), 124(55), 133(50), 98(30)
Sotalol	1.092	296	281(100), 239(65), 296(20)
Timolol	1.121	370	340(70), 325(70), 138(90), 98(100), 228(20)
Atenolol	1.134	290	275(100), 290(10), 164(10), 98(15), 124(15),

[a] RRT = relative t_R to propranolol.

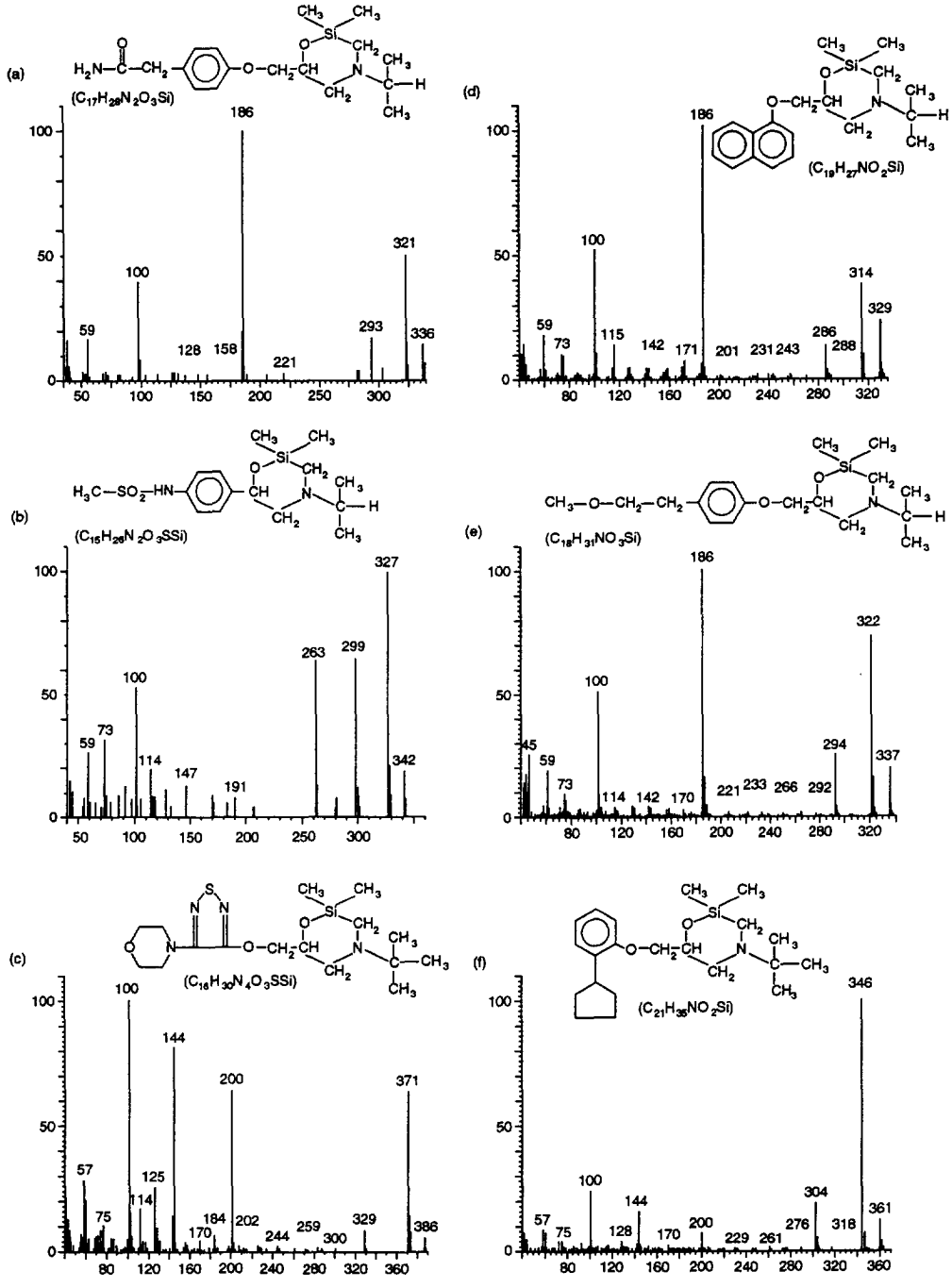

Fig. 5. Full mass spectra for cyclised silyl derivatives of (a) atenolol, (b) sotalol, (c) timolol, (d) propranolol, (e) metoprolol, (f) penbutolol, (g) alprenolol, (h) bamethane, (i) oxprenolol and (j) pindolol.

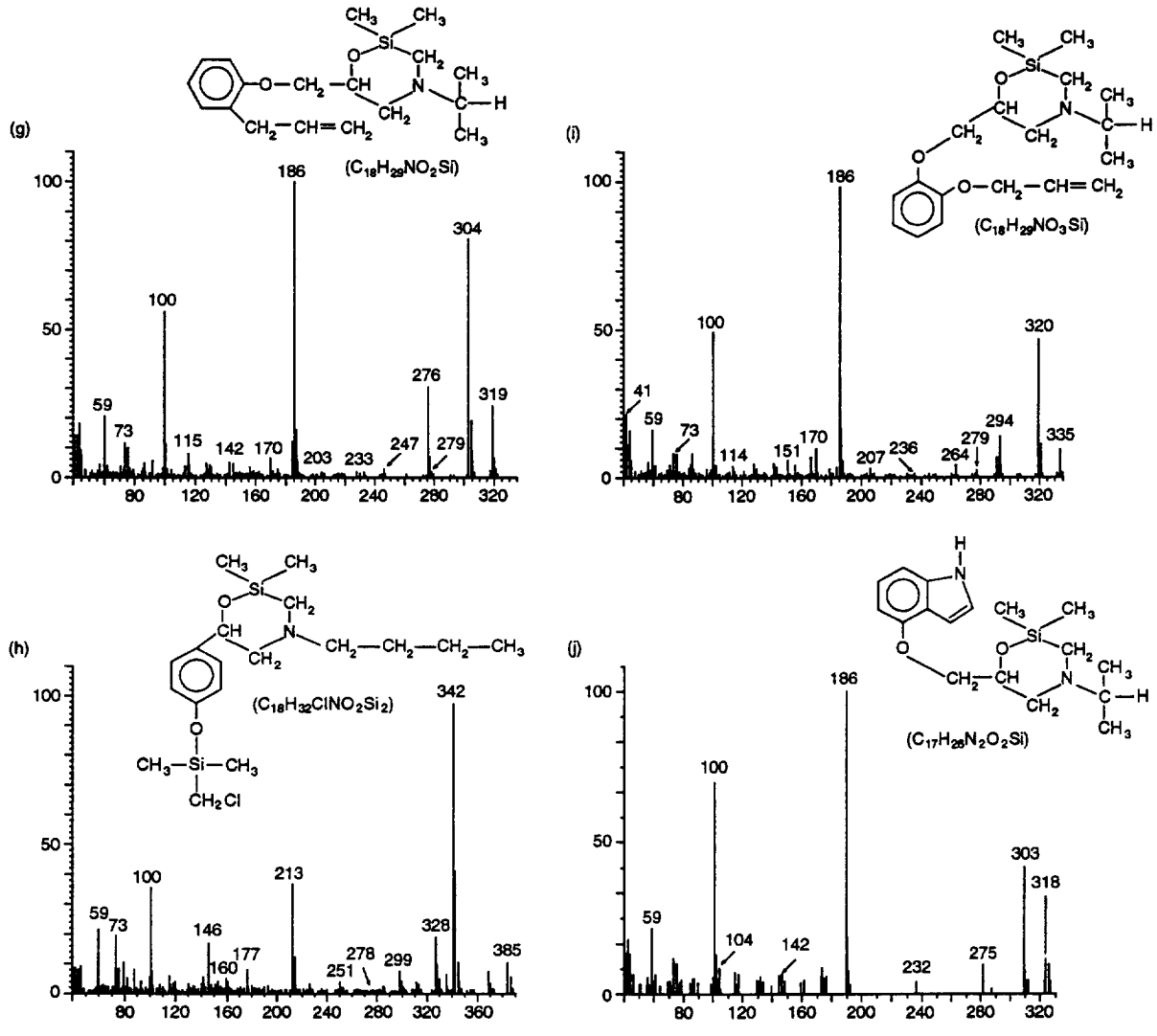

Fig. 5. (*continued*)

fragmentation. Greater specificity is due to high abundance and stability of higher mass ions (Fig. 6). Also, these derivatives have been found to be stable at room temperature for at least three days and have been reported [3] to be stable for over a week at 4°C.

Bamethane is not a prescribed drug in Australia and therefore it would make a suitable internal standard in the screening procedure. Our work with bamethane indicates that it derivatised fully to the cyclised silyl derivative (Table 1 and Fig. 5) using the derivatisation procedure as described. Using the experimental GC conditions, bamethane coelutes with propranolol and penbutolol. Further work on the extraction efficiency of bamethane, using the column solid-phase extraction methods as described, is required to assess bamethane's suitability as an internal quantitative standard. Bamethane may not be a suitable internal standard outside Australia where bamethane may be a commonly prescribed drug. In such cases, a different β-blocker which is not prescribed in that country may be more suitable as internal standard.

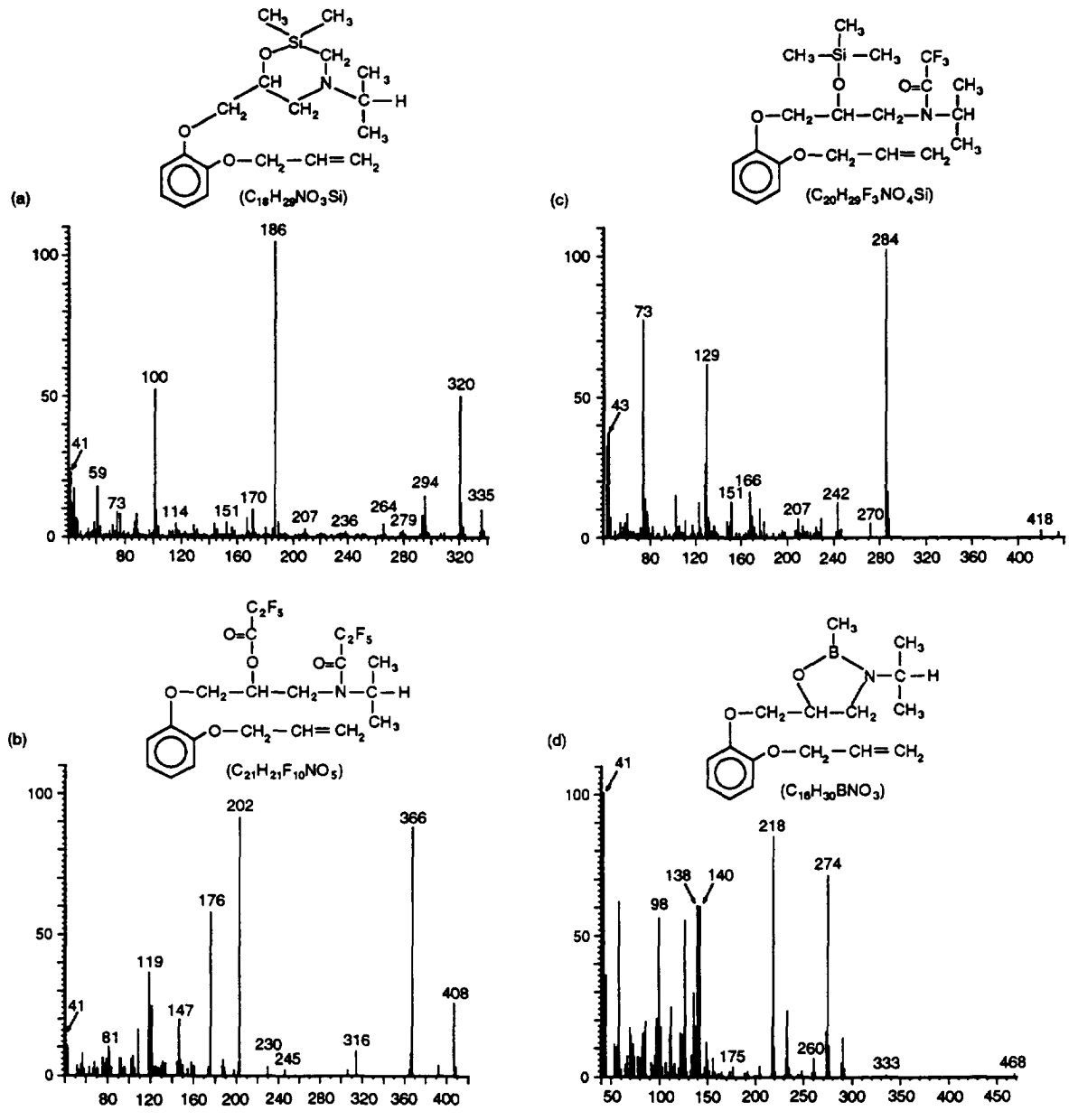

Fig. 6. Full mass spectra for (a) cyclised silyl, (b) PFP, (c) TMS-TFA and (d) cyclic methylboronate derivatives of oxprenolol.

4. Conclusion

The preferred technique for analysis of β-blockers in human post mortem whole blood and urine involves solid-phase extraction using either XtrackT XRDAH515 or Bond Elut Certify cartridges, derivatisation with CMDMCS to form cyclised silyl derivatives and analysis by GC–MS using combined SIM. This method has been successfully applied in the Forensic Science Laboratory of the Chemistry Centre (WA) for the screening and confirmation of β-blockers in forensic case work.

The preferred technique for analysis of β-blockers in equine urine involves dual column solid-phase extraction using Sep-Pak C_{18} and XtrackT XRDAH515 cartridges, derivatisation with CMDMCS to form cyclised silyl derivatives and analysis by GC–MS using combined SIM.

Acknowledgments

This work was published with the approval of Director CCWA and Mr D.A. McCann (Perth Coroner). The authors are grateful for the gifts of β-blockers made by their respective manufacturers.

References

[1] C.G. Hammar, Biomed. Mass Spectrom., 5 (1978) 25.
[2] K.J. Hoffmann, A. Arfwidsson, K.O. Borg and I. Skånberg, Biomed. Mass Spectrom., 5 (1978) 634.
[3] M.C. Dumasia and E. Houghton, J. Chromatogr., 564 (1991) 503.
[4] S.H. Wan, R.F. Maronde and S.B. Matin, J. Pharm. Sci., 67 (1978) 1340.
[5] G.P. Cartoni, M. Ciardi, A. Giarruso and F. Rosati, J. High Resolut. Chromatogr., 11 (1988) 528.
[6] M.S. Leloux, E.G. De Jong and R.A.A. Maes, J. Chromatogr., 488 (1989) 357.
[7] J. Zamecnik, J. Anal. Toxicol., 14 (1990) 132.
[8] L. Soltés, Biomed. Chromatogr., 3 (1989) 139.
[9] D.S. Lho, J.K. Hong, H.K. Paek, J.A. Lee and J. Park, J. Anal. Toxicol., 14 (1990) 77.
[10] M.S. Leloux and R.A.A. Maes, Biomed. Environ. Mass Spectrom., 19 (1990) 137.
[11] L.K. Pannell, B.M. Thomson and L.F. Wilkinson, J. Anal. Toxicol., 6 (1982) 193.
[12] A.M. Duffield, S. Wise, K. Keledjian and C.J. Suann, in D.J. Honey and V.J. McLinden (Editors), Proceedings of the 27th International Meeting of The International Association of Forensic Toxicologists, Perth, Western Australia, October 19–23, 1990, p. 141.
[13] F.T. Delbeke, M. Debackere, N. Desmet and F. Maertens, J. Chromatogr., 426 (1988) 194.
[14] F.T. Delbeke, M. Debackere, N. Desmet and F. Maertens, J. Pharm. Biomed. Anal., 6 (1988) 827.
[15] G. Musch, Y. Buelens and D.L. Massart, J. Pharm. Biomed. Anal., 7 (1989) 483.
[16] A.M. Duffield, S. Wise, K. Keledjian and C.J. Suann, J. Chromatogr., 518 (1990) 215.
[17] H. Maurer and K. Pfleger, J. Chromatogr., 382 (1986) 147.
[18] D.W. Roberts, R.J. Ruane and I.D. Wilson, J. Pharm. Biomed. Anal., 7 (1989) 1077.
[19] G.L. Hoyer, J. Chromatogr., 427 (1988) 181.
[20] K.P. Devi, K.V.R. Rao and S.K. Baveja, J. Chromatogr., 434 (1988) 265.
[21] A. Tracqui, P. Kintz, J. Himber, A.A.J. Lugnier and P. Mangin, Forensic Sci. Int., 38 (1988) 37.
[22] J.B. Fourtillan, M.A. Lefebvre, J. Girault and P. Courtois, J. Pharm. Sci., 70 (1981) 573.
[23] P.M. Harrison, A.M. Tonkin and A.J. McLean, J. Chromatogr., 339 (1985) 429.
[24] P.M. Harrison, A.M. Tonkin, C.M. Cahill and A.J. McLean, J. Chromatogr., 343 (1985) 349.
[25] M.J. Bartek, M. Vekshteyn, M.P. Boarman and D.G. Gallo, J. Chromatogr., 421 (1987) 309.
[26] G. Lubli, C. Neri, S. Chiminazzo, L. Bonizzato and M. Marigo, in D.J. Honey and V.J. McLinden (Editors), Proceedings of the 27th International Meeting of The International Association of Forensic Toxicologists, Perth, October 19–23, 1990, p. 258.
[27] A.C. Moffat, J.V. Jackson, M.S. Moss and B. Widdop (Editors), Clarke's Isolation and Identification of Drugs, The Pharmaceutical Press, London, 1986, p 331–1027.
[28] R.C. Baselt and R.H. Cravey (Editors), Disposition of Toxic Drugs and Chemicals in Man, 3rd Ed, Year Book Medical Publishers, Chicago, IL, 1989, p. 27–802.