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Liquid chromatography–mass spectrometry for the determination of medetomidine and other anaesthetics in plasma

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ABSTRACT

A liquid chromatographic–atmospheric pressure chemical ionization mass spectrometric method is presented for the simultaneous determination of medetomidine and other anaesthetic drugs in solutions and dog plasma. The drugs examined were flumazenil, butorphanol, atropine, ketamine, xylazine, medetomidine, atipamezole and midazolam. The separation was carried out on a reversed-phase column using methanol–0.1 *M* ammonium acetate (3:2) as eluent.

INTRODUCTION

Medetomidine, 4-[1-(2,3-dimethylphenyl)ethyl]-1*H*-imidazole hydrochloride, is used as an analgesic sedative for animals. It is a full agonist at both pre- and postsynaptic α_2-adrenoreceptors. Human use for premedication is under investigation. Medetomidine has no significant absorption at wavelengths longer than 220 nm and lacks native fluorescence, which make it difficult to develop a chromatographic assay method for the drug and its metabolites. Vuorilehto *et al.* [1] reported its gas chromatographic–mass spectrometric assay in serum. Although the method is very sensitive, the need for derivatization with pentafluorobenzoyl chloride makes it tedious.

Medetomidine and related sedatives and anaesthetics used in human and animals are shown in Fig. 1. Atipamezole, 4-(2-ethyl-2,3-dihydro-1*H*-inden-2-yl)-1*H*-imidazole, is an α_2-adrenoreceptor antagonist, while xylazine, 2-(2,6-dimethylphenylamino)-4*H*-5,6-dihydro-1,3-thiazine, is an agonist as well as medetomidine. Butorphanol tartrate, 17-(cyclobutylmethyl)morphinan-3,14-diol tartrate, is a narcotic agonist–antagonist analgesic, and is available for use in humans and animals. Ketamine, 2-(*o*-chlorophenyl)-2-(methylamino)cyclohexanone hydrochloride, is an anaesthetic. Midazolam, 8-chloro-6(*o*-fluorophenyl)-1-methyl-4*H*-imidazol-[1,5-*a*] [1,4]benzodiazepine, is a benzodiazepine agonist and flumazenil, ethyl 8-fluoro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazol[1,5-*a*] [1,4]benzodi-

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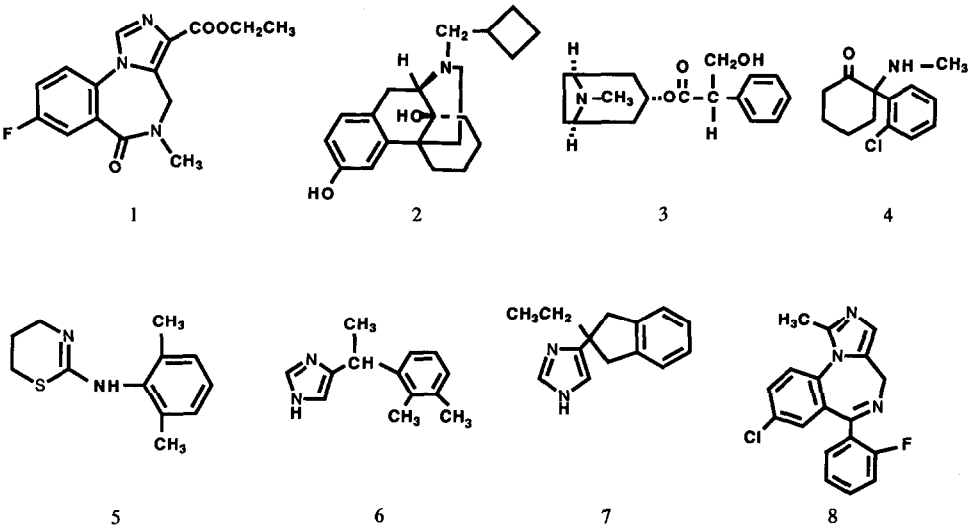

Fig. 1. Structures of the anaesthetic drugs studied. **1** = Flumazenil; **2** = butorphanol; **3** = atropine; **4** = ketamine; **5** = xylazine; **6** = medetomidine; **7** = atipamezole; 8 = midazolam.

azepine-3-carboxylate, a benzodiazepine antagonist. Atropine is a common anticholinergic drug.

These drugs have recently been used in combination with medetomidine or used for premedication for balanced anaesthesia and sedation in veterinary science. Assay methods by means of gas chromatography (GC) or high-performance liquid chromatography (HPLC) have been reported for xylazine [2], ketamine [3,4], midazolam [5], atipamezole [6] and butorphanol [7]. A suitable simultaneous method for the determination of the drugs in biological samples is required.

HPLC is one of the most widely used methods for the determination of drugs in biological samples. Among the various HPLC techniques, liquid chromatography–atmospheric pressure chemical ionization mass spectrometry (LC–APCI-MS) shows promise in various field [8]. This paper describes an LC–APCI-MS assay for the simultaneous determination of the drugs in solution and dog plasma.

EXPERIMENTAL

Instrumentation

The apparatus used was a Hitachi L-6200 HPLC instrument with a Rheodyne Model 7125 valve, connected to a Hitachi M1000 mass spectrometer–computer system through the APCI interface. The nebulizer and vaporizer temperatures were 260 and 399°C, respectively. The chromatographic separation was carried out on a column of octadecylsilylsilica (Hitachi gel 3056, 150 mm × 4.6 mm I.D.) using methanol–0.1 *M* ammonium acetate (3:2) as the eluent at room temperature.

Reagents

The drugs used were obtained from commercial sources as follows: medetomidine hydrochloride (Domitor; Famos Group, Turku, Finland); butorphanol tartrate (Stadol; Bristol–Myers Squibb, Tokyo, Japan); midazolam (Dormicum; Yamanouchi Pharmaceutical, Tokyo, Japan); xylazine hydrochloride (Celactal; Bayer, Tokyo, Japan); atipamezole hydrochloride (Antisedan; Famos Group); atropine sulphate (Tanabe, Osaka, Japan); ketamine hydrochloride (Ketaral; Sankyo, Tokyo, Japan); flumazenil (Hoffman-La Roche, Nutley, NJ, USA).

Water was distilled and passed through a Milli-Q purification system (Millipore, Bedford, MA, USA). All other chemicals were of analytical-reagent grade.

Sample preparation

A standard solution was prepared so as to contain 0.1, 1.0, 0.5, 0.5, 2.0, 1.0, 5.0 and 0.5 mg/ml of

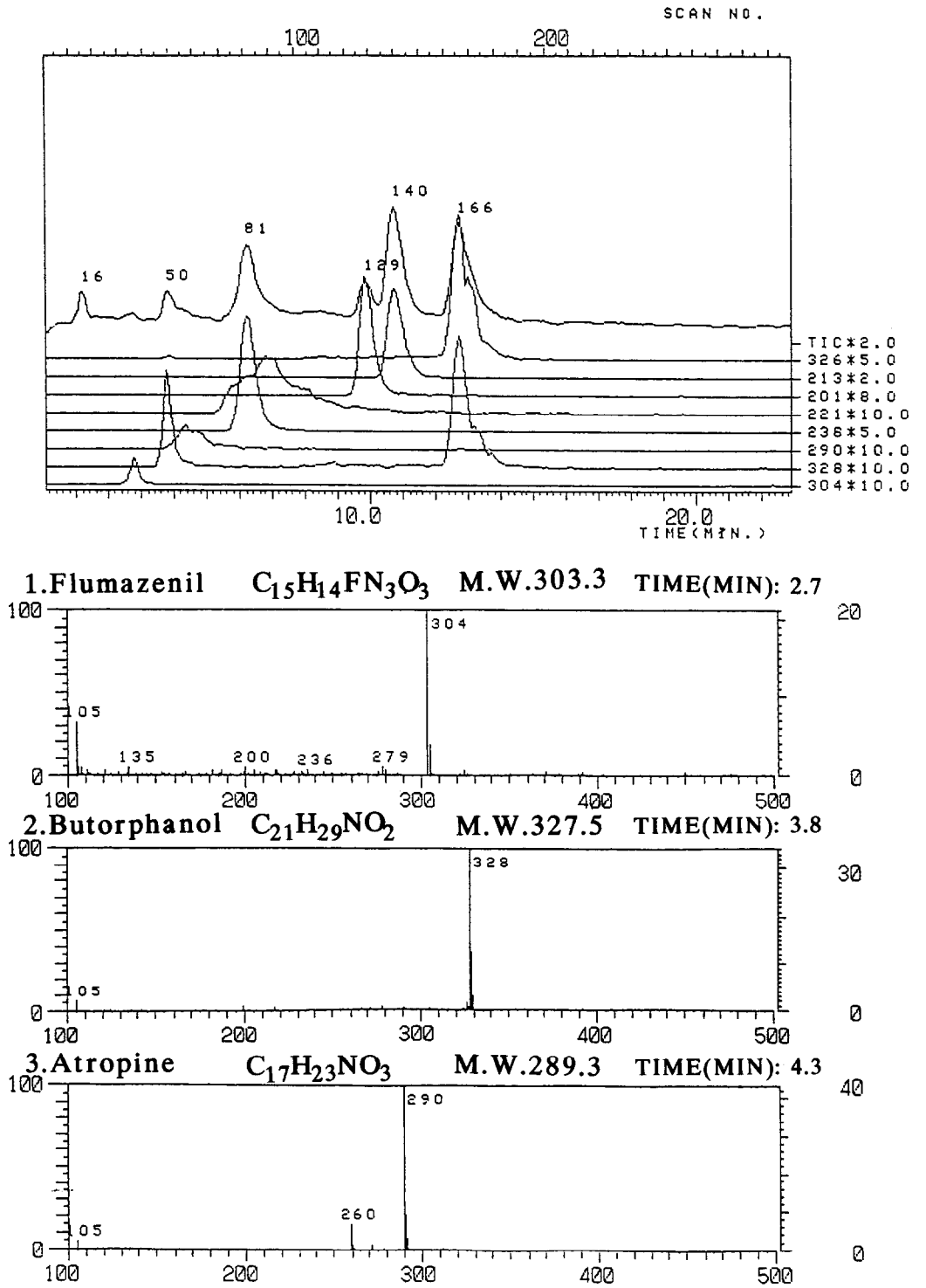

Fig. 2.

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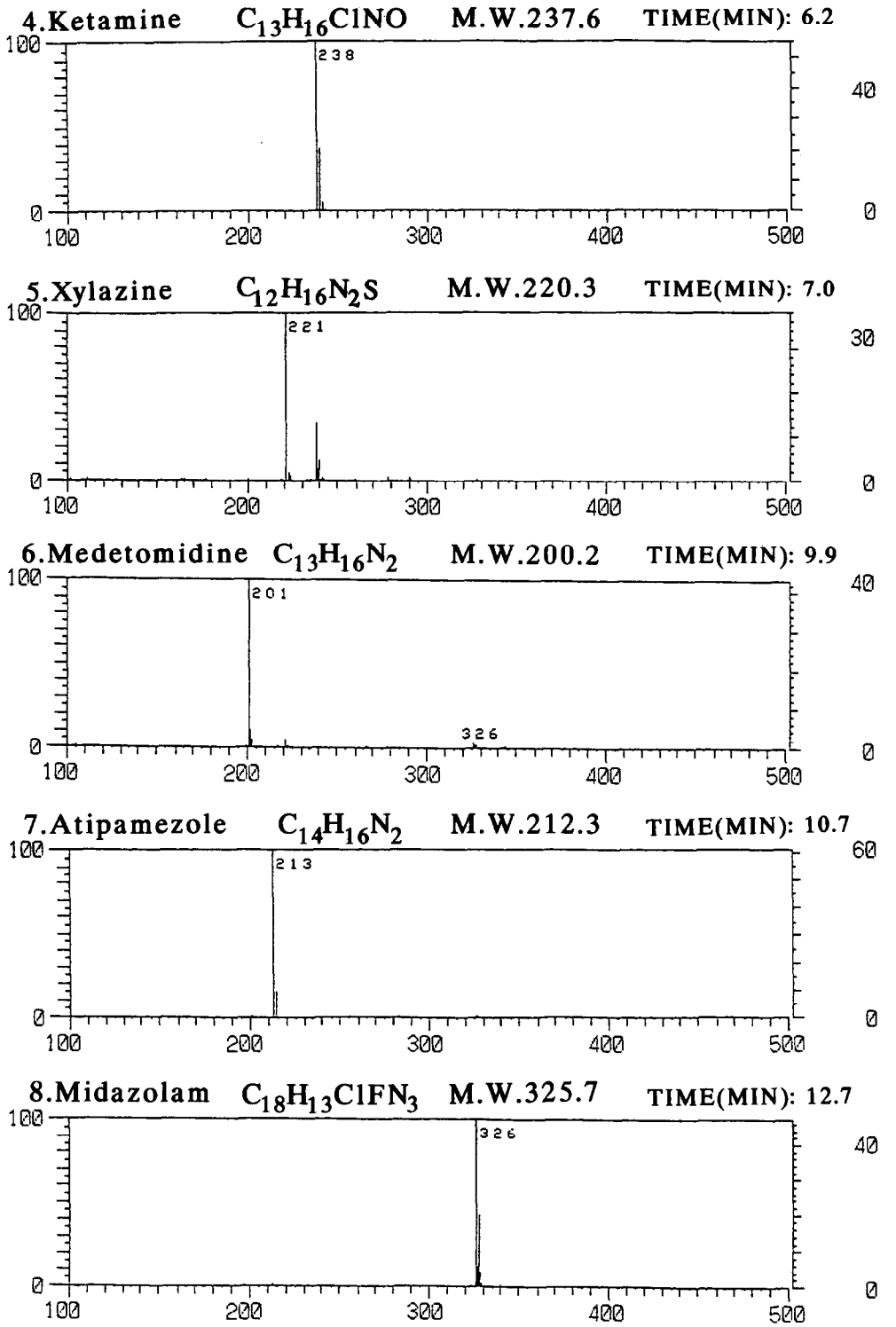

Fig. 2. Mass chromatograms of a mixture of the drugs and mass spectra scanned at the peak tops of the mass chromatograms. The HPLC conditions are described in the text. The mass spectrometer was scanned from m/z 100 to 500 at 4 s per scan. M.W. = Molecular mass. Numbers at right-hand side axis of mass chromatogram indicate m/z multiplied by a factor. TIC = total ion chromatogram.

flumazenil, butorphanol, atropine, ketamine, xylazine, medetomidine, atipamezole and midazolam, respectively. A 20-μl volume of the solution was injected into the HPLC system.

A 200-μl volume of dog plasma spiked with 20 μl of the standard solution was applied to a Sep-Pak C_{18} cartridge pretreated with water, methanol and 0.1 *M* ammonium acetate. After washing the column with 0.1 *M* ammonium acetate, the sample was eluted with methanol–0.1 *M* ammonium acetate (3:1) and the eluate was evaporated to dryness under reduced pressure. The residue was dissolved in 200 μl of the eluent and 20 μl of the solution were injected into the HPLC system.

RESULTS AND DISCUSSION

Mass chromatograms and mass spectra of a standard solution of the eight drugs obtained using the LC–APCI-MS system are shown in Fig. 2. The well resolved chromatograms were obtained with methanol–0.1 *M* ammonium acetate (3:2) as the eluent at a flow-rate of 1 ml/min in 15 min. Although the peak shapes of **3** and **5** were poor, the mass spectra showed that they were well separated. In the LC–APCI-MS system, quasi-molecular ions $[M+H]^+$ of these drugs were observed as base peaks.

Solutions containing 0–500 ng of the drugs were injected and the corresponding peak areas were integrated. The limits of the detection were 10–50 pg per injection (signal-to-noise ratio = 3). The calibration graphs were linear and reproducible. The relationship calculated between the peak area (y) and the concentration of the drugs (x) and the correlation coefficients (r) were as follows: **1**, $y = 2.59x + 0.36$ ($r = 0.9999$); **2**, $y = 1.84x - 0.11$ ($r = 0.9995$); **3**, $y = 1.38x + 0.26$ ($r = 0.9999$); **4**, $y = 2.21x + 0.29$ ($r = 0.9995$); **5**, $y = 2.09x + 0.54$ ($r = 0.9999$); **6**, $y = 3.17x + 0.86$ ($r = 0.9999$); **7**, $y = 1.65x - 0.43$ ($r = 0.9998$); and **8**, $y = 5.39x - 0.37$ ($r = 0.9994$).

Mass chromatograms of an extract of dog plasma spiked with the drugs are shown in Fig. 3. The peaks were separated satisfactorily to allow the determination of each component. The recoveries of the drugs from plasma were 84.5, 73.1, 106.7, 84.0,

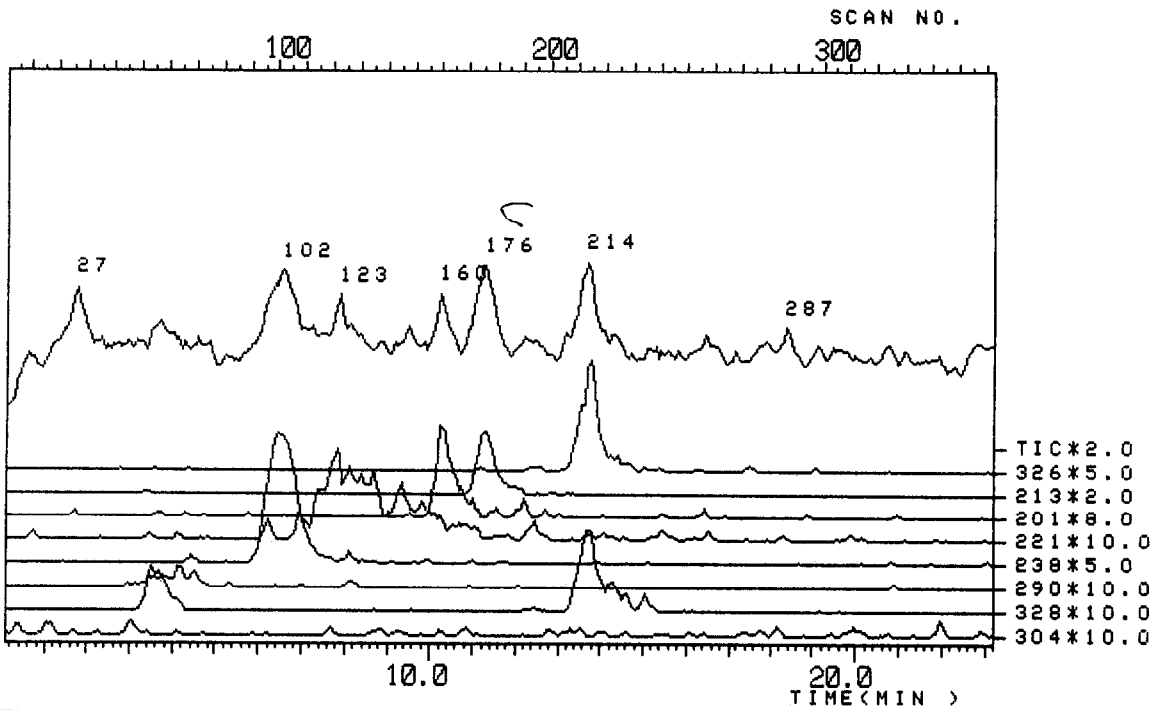

Fig. 3. Mass chromatograms of dog plasma spiked with the eight drugs.Numbers at right indicate m/z multiplied by a factor. TIC = total ion chromatogram.

101.9, 97.4, 95.4 and 96.1% (n = 3) for **1**, **2**, **3**, **4**, **5**, **6**, **7** and **8**, respectively.

The method can be also used for the identification and determination of metabolites of medetomidine and the other drugs in plasma. Work on this aspect is in progress.

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