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Original works

Detection of methadone in human hair by gas chromatography/mass spectrometry

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Summary. Determination of methadone in human hair by gas chromatography/mass spectrometry was described. Helium as carrier gas, a 30-m bonded phase-fused silica DB-1 capillary column and splitless injection at 230°C temperature were used. The concentrations of methadone and its metabolites were measured in addition by radioimmunoassay (RIA). Both methods GC/MS and RIA showed the presence of methadone in human hair.

Key word: Methadone, in human hair

Zusammenfassung. In dieser Arbeit wurde die Bestimmung von Methadon in menschlichen Haaren mittels Gaschromatographie/Massenspektrometrie (GC/MS) beschrieben. Als Trägergas wurde Helium benützt; die Säule war eine Kapillarsäule DB-1 un die Injektion eine Splitlos-Injektion, bei einer Verdampfungstemperatur von 230°C. Die Methadonkonzentrationen in den Haaren wurden auch mittels Radioimmunoassay (RIA) durchgeführt. Beide Methoden GC/MS und RIA zeigen das Vorhandensein des Methadons im menschlichen Haar.

Schlüsselwort: Methadon, im menschlichen Haar

Introduction

Recently, we have described the determination of methadone in human hair by radioimmunoassay (Balabanova and Wolf 1988). The method described is sensitive, simple, and rapid. The determination is practicable without pretreatment of the samples.

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However, the antibody used react with methadone and its metabolites. The concentrations measured are the sum of methadone, methadol, and normethadol. Both metabolites are present in plasma in small amount only.

In forensic science the determination of drugs is obliged to be perfomed by two different methods. Consequently, in this study we described a second method specific for the determination of methadone alone in hair by gas chromatography/ mass spectrometry (GS/MS).

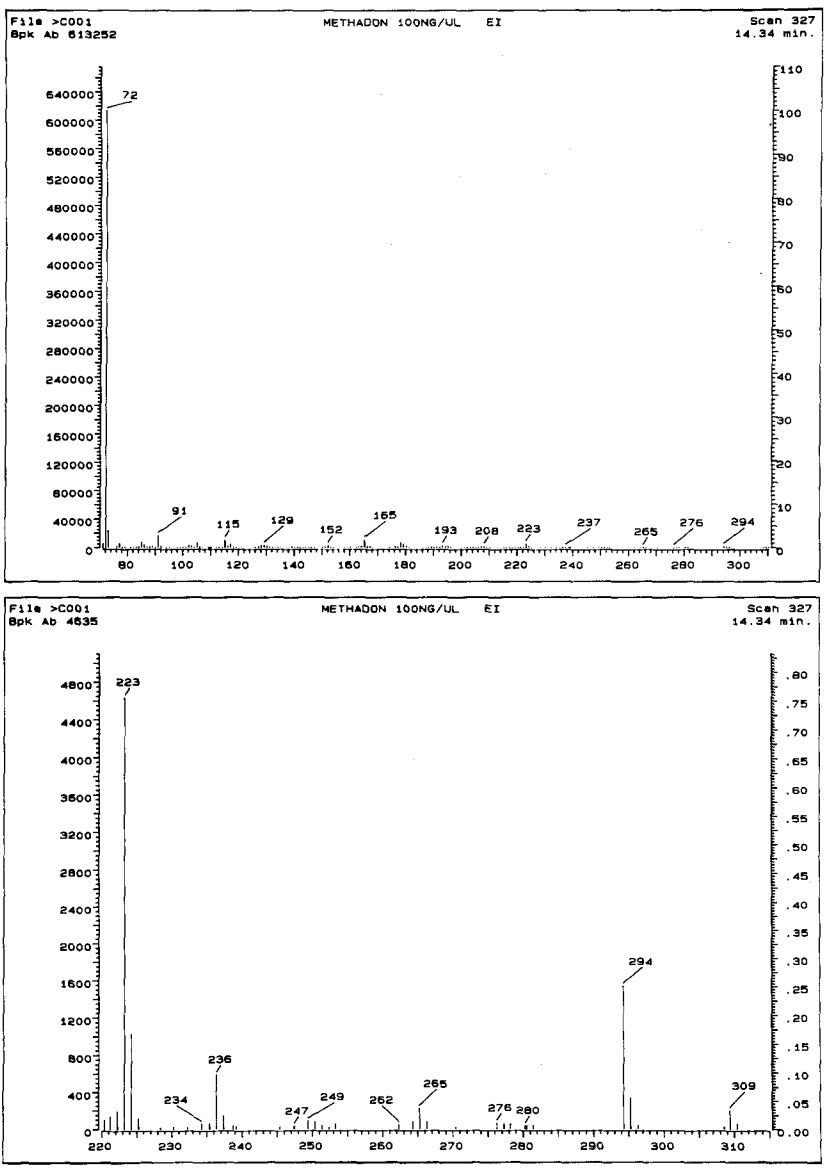

Fig. 1. Methadone-selected ions

Material and methods

Hair from the head, pubic and axillary hair were obtained simultaneously from seven patients receiving a daily maintenance dose of methadone (30 mg/day).

After washing of the hair, 50 mg was crushed with 1 ml 0.1 *M* HCl and incubated overnight at 45°C. The acid extracts were neutralized with 0.1 ml of 1 *M* NaOH and diluted with phosphate buffer up to pH 8.2, and then extracted with chloroform and injected into the GC/MS.

GC/MS

GC/MS analysis was performed on a Hewlett Packard 5890 A gas chromatograph connected to a Hewlett Packard 5988 A mass spectometer. Helium was used as a carrier gas at a flow rate of 2 ml/min. A 30-m bonded phase fused-silica DB 1 capillary column, 0.32 mm i.d. was used. Splitless injection was performed at 230°C evaporator temperature and 50°C oven temperature. After this, the oven temperature was raised to 200°C at a rate of 25°C/min and maintained for 6 min. The ionization mode was electron impact, ionizing voltage; 70 eV. The lowest detection limit of methadone under this condition was 6 pg injection.

RIA

In addition, the concentrations of methadone and its metabolites were determined by radioimmunoassay (Biermann, FRG). The hair extract obtained as described above were diluted with phosphate buffer at pH 7.4. The antiserum was prepared in sheep, the tracer was I^{125}-labeled methadone. The lower limit of detection was 2.5 ng/mg hair. The intra- and inter-assay coefficients of variation were 7.9% ($n = 7$) and 9.2% ($n = 37$). The cpm of the unknown samples were converted to nanogram equivalent per milliliter by use of the calibration curve, and then converted to ng/mg hair.

Results

The mass spectrum of methadone alone with molecular ion at m/e 309 and mass fragments at m/e 72, 223, 294 and the ion chromatogram are depicted in Figs. 1 and 2. As shown in Fig. 1, the best suitable m/e values to the proof of methadone are 72, 223, and 294.

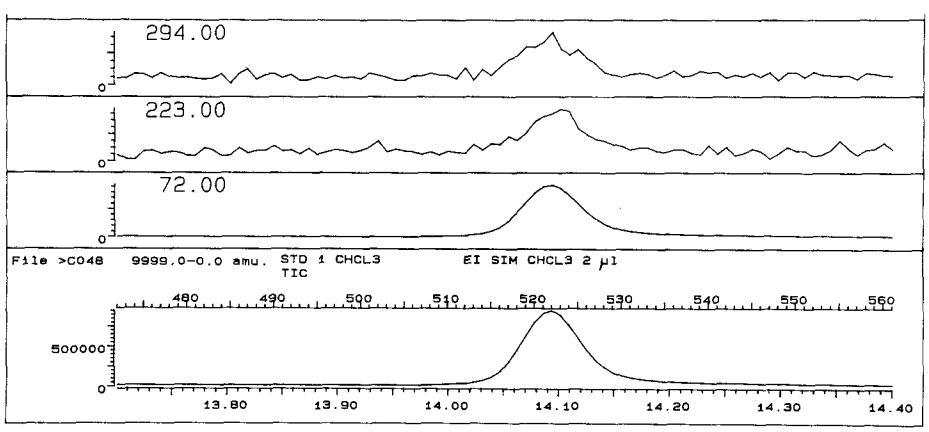

Fig. 2. Methadone-ions chromatograms (Methadone standard)

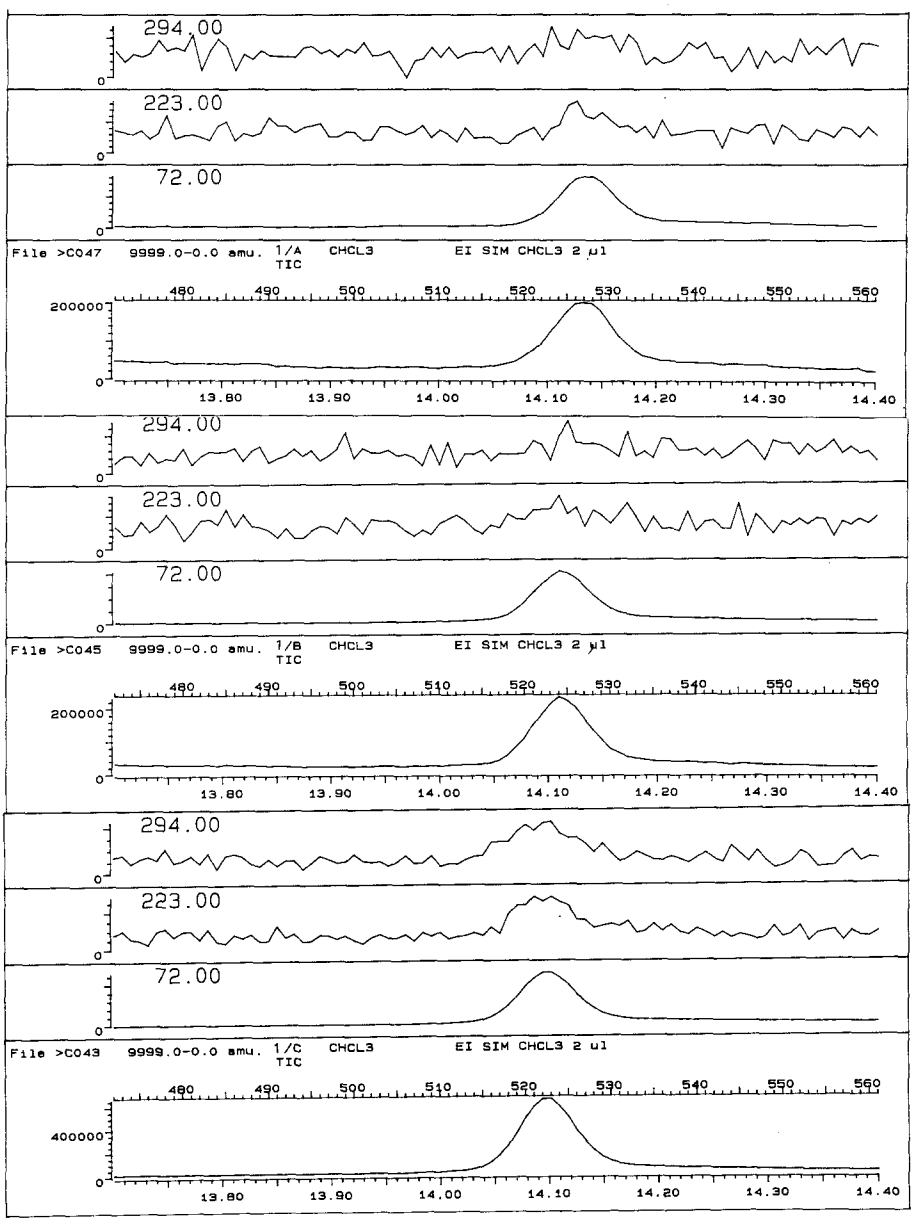

Fig. 3a–c. Methadone-selected ions and chromatograms. **a** Hair of the head, **b** pubic hair, and **c** axillary hair obtained from patient no. 1

The mass spectrum obtained from hair extracts showed the same ions and clean and symmetrical peaks of the ion chromatogram as the standard (Fig. 3a, b). The highest concentrations were found in axillary hair followed by pubic hair and hair of the head. These results are in accordance with the results obtained by RIA (Table 1).

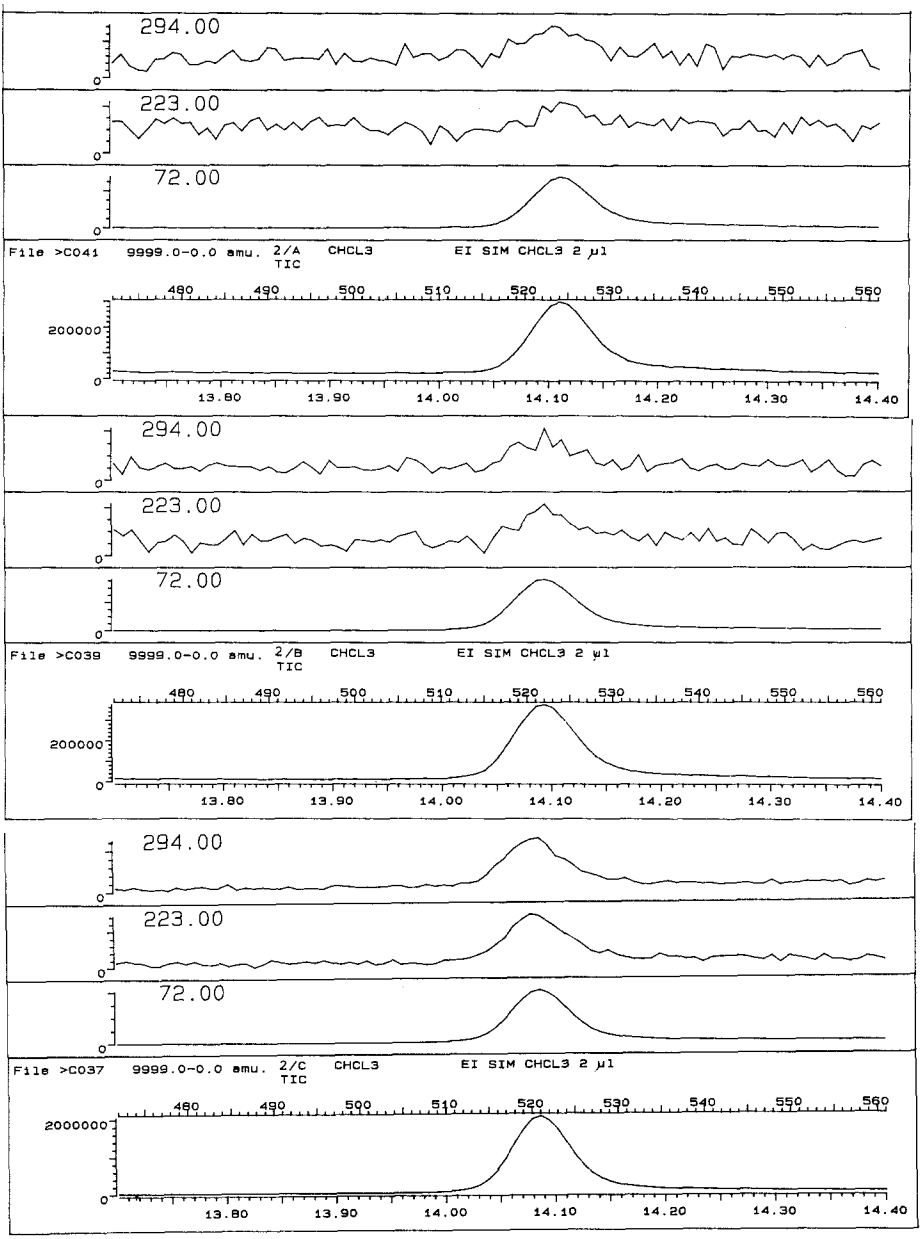

Fig. 4. Methadone-selected ions and chromatograms. **a** Hair of the head, **b** Pubic hair, and **c** axillary hair obtained from patient no. 2

Comparison of peak areas from known quantities of standard with those from hair samples extracted in an identical manner make it possible to estimate the methadone concentrations in the samples.

The mass spectrum obtained from hair of nonaddict persons is shown in Fig. 5.

Table 1. Concentrations of methadone and its metabolites in hair of the head, pubic and axillary hair measured by RIA

	Methadone concentrations (ng/mg hair) in:		
Patient no.	Hair of the head	Pubic hair	Axillary hair
1	1.4	2.1	3.7
2	2.9	3.9	6.9
3	2.0	2.9	3.4
4	0.7	1.4	2.5
5	1.0	3.1	4.2
6	0.5	1.0	2.8
7	1.0	1.6	2.8
Mean values:	1.4	2.3	3.8

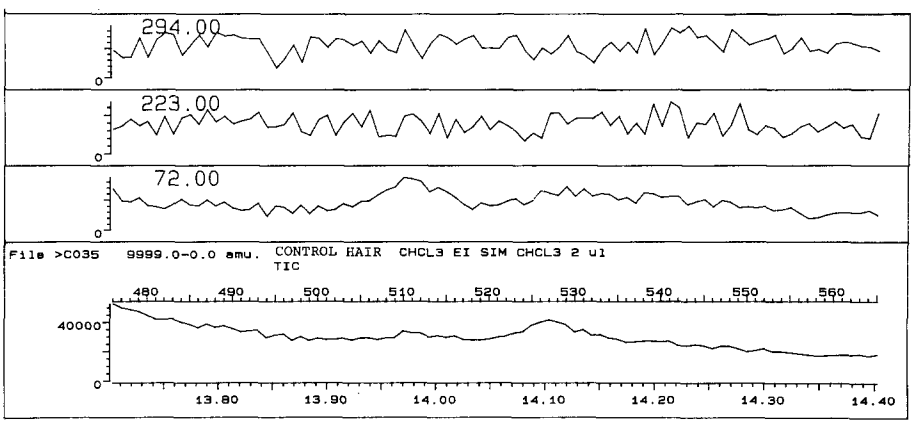

Fig. 5. Chromatogram obtained from hair of a nonaddict person

Discussion

Determination of methadone in biologic fluid by GC/MS has been documented (Hachey et al. 1977; Sullivan et al. 1975). The presence of methadone in human hair was demonstrated by radioimmunologic method (Balabanova and Wolf 1988) but not by GC/MS.

This study described the determination of methadone alone in human hair by GC/MS. The results obtained were compared with these obtained by RIA. The concentrations measured by RIA are the sum of methadone and its metabolites. On the contrary, the GC/MS procedure is designed specifically for the determination of methadone alone or its metabolites and use selective ion monitoring in the electron impact mode. Thus both methods, GC/MS and RIA, showed the presence of methadone in hair and consequently fulfill the requirements in forensic science.

References

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