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Short communication

Evaluation of urinary dihydrocodeine excretion in human by gas chromatography–mass spectrometry

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Abstract

Urinary metabolic pattern after the therapeutic peroral dose of dihydrocodeine tartrate to six human volunteers has been explored. Using the GC–MS analytical method, we have found that the major part of the dose administered is eliminated via urine within the first 24 h. However, the analytical monitoring of dihydrocodeine and its metabolites in urine was still possible 72 h after the dose was administered. The dihydrocodeine equivalent amounts excreted in urine in 72 h ranged between 32 and 108% of the dose, on average 62% in all individuals. The major metabolite excreted into urine was a 6-conjugate of dihydrocodeine, then in a lesser amount a 6-conjugate of nordihydrocodeine (both conjugated to approximately 65%). The *O*-demethylated metabolite dihydromorphine was of a minor amount and was 3,6-conjugated in 85%. Traces of nordihydromorphine and hydrocodone were confirmed as other metabolites of dihydrocodeine in our study. This information can be useful in interpretation of toxicological findings in forensic practice.

Keywords: Dihydrocodeine

Nomenclature

DHC	dihydrocodeine
DHM	dihydromorphine
NDHC	nordihydrocodeine
NDHM	nordihydromorphine
HC	hydrocodone
MO–D3	morphine(*N*-methyl-D3)
TMS	trimethylsilyl derivative
MSTFA	*N*-methyl-*N*-trimethylsilyltrifluoroacetamide

1. Introduction

The pharmacological effects of dihydrocodeine are very similar to codeine [1]. Dihydrocodeine has been therapeutically applied for decades, nevertheless its metabolism has been studied less extensively than in the case of codeine. It is therapeutically used as a cough suppresant and mild analgesic in single doses of 10–60 mg up to a total daily dose of 80–240 mg

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in adults [1]. In some countries it is prescribed as a substitute drug in the treatment of opiate addicts [2]. It can be abused also as a component of some illicit preparations (e.g. ''Brown'' [3]) and fatal intoxications are often due to polydrug use [4]. A wide individual variability in metabolism and pharmacokinetics can be observed during various opiates use.

Recently, it has been published that dihydrocodeine undergoes biotransformation steps analogous to codeine: *O*-demethylation to dihydromorphine, *N*-demethylation to nordihydrocodeine and nordihydromorphine, and conjugation of parent drug and hydroxylated metabolites with glucuronic acid. Individual variability of metabolism within humans has been established due to various enzyme activities, especially cytochrome P450 2D6 being responsible for *O*-demethylation of dihydrocodeine [5–8].

Various methods were applied to explore the metabolic profile of dihydrocodeine: HPLC [2,8], MECC [6,7], GC–MS–MS [5]. The goals of this experimental study have been to contribute to further elucidation of the urinary metabolic pattern after a single peroral dose of dihydrocodeine to human volunteers using GC–MS method. The detailed knowledge of biotransformation and disposition of xenobiotics in man can play an important role in the interpretation of analytical toxicological findings in various forensic or clinical situations.

2. Experimental

2.1. Reference standard substances, chemicals

All solvents and reagents were of analytical grade quality. One-hundred and thirty milligrams Bond Elut Certify extraction columns (1211–3050) were obtained from Varian, Harbor City, CA, USA. Silylating reagents MSTFA, ammonium iodide, and 1,2-ethanedithiobis(trimethylsilane) were purchased from Fluka Chemie, Buchs, Switzerland.

The following reference standard substances were used for GC–MS analyses: Dihydrocodeine tartrate, M_r 451.5, Napp Research Centre, Cambridge, UK. Dihydromorphine base, M_r 287.4, Napp Research Centre, Cambridge, UK. Dihydronorcodeine trifluoroacetate monohydrate, M_r 419.4, Lipomed, Arlesheim, Switzerland.

Dihydronormorphine hydrochloride, M_r 309.4, Lipomed A.G., Arlesheim, Switzerland. Hydrocodone tartrate, M_r 449.5, Sigma, St. Louis, MO, USA. Morphine(*N*-methyl-D3) monohydrate, M_r 306.4, Lipomed, Arlesheim, Switzerland.

2.2. Drug administration and urine sampling

Six human volunteers gave their informed consent to participate in the study. Just before drug administrations the urine morning voids (blank) were collected to be analyzed for absence of opiates. Volunteers without any medication and poppy seeds in diet 3 days before, fasting since evening, were administered the peroral therapeutic dose of 0.25 mg dihydrocodeine tartrate/kg body weight. Excreted urine fractions were sampled at given time intervals during 72 h. The sampling intervals (in hours) after drug administration were: 0–2, 2–6, 6–10, 10–16, 16–22, 22–30, 30–38, 38–46, 46–55, 55–64 and 64–72. Urine samples were stored at −20°C until analyses.

2.3. Hydrolysis of conjugates, extraction, silylation

For acid hydrolysis, 1 ml of urine sample with 0.25 ml concentrated hydrochloric acid were incubated at 110°C for 60 min using tightly capped glass tubes. The time of hydrolysis was optimized to achieve the maximum yield of hydrolytic reaction breaking the conjugation bonds. After cooling, sample pH were adjusted to values between 8 and 9 with Tris buffer and then samples were mixed with deuterated morphine as internal standard (200 ng in 10 μl methanol). To evaluate the degree of conjugation, samples were also pretreated omitting the hydrolysis step. The solid-phase extraction on mixed copolymeric material (Bond Elut Certify) was performed according to the commercial manual enclosed in the package. After eluent evaporation, dry eluates were silylated with 100 μl silylating mixture by heating at 80°C for 20 min and 1 μl was analyzed using the GC–MS method. The mixture for efficient silylation of molecules with rather problematic structural groups (normetabolites, hydrocodone) consisted of MSTFA and ammonium iodide stabilized with

1,2-ethanedithiobis(trimethylsilane) in the mass ratio 1000:2:3.

2.4. GC–MS analyses

The instrument used was a HP GC–MSD 6890-5973, splitless injector at 250°C, auxiliary 270°C, capillary HP5-MS 30 m×250 μm×0.25 μm, carrier gas He at constant flow 1 ml/min, oven temperature programmed from 85°C 2 min, 30°C/min till 220°C, then 3°C/min till 260°C, then 15°C/min till 280°C, then held for 3.5 min, time of analysis 25 min. MSD was working either in standard electron impact scan mode in the range 45–550 m/z or in SIM mode. In the scan mode, used for the sake of identification, the limit of detection was 7 ng/ml or lower (signal-to-noise ratio 3).

Quantitation using the internal standard method in SIM mode was based on quantitation ion peak area measurements employing six level calibrations (5, 100, 500, 1000, 2000 and 3000 ng/ml). The ions for quantitation of silylated analytes in SIM mode were: DHC m/z=373, DHM m/z=431, NDHC m/z=431, NDHM m/z=489, HC m/z=371, internal standard MO–D3 m/z=432. Performing sample pretreatment as described above, run to run variations (n=6) were determined at a low concentration level of 10 ng/ml to be 8% for DHC, 2% for DHM, 15% both for NDHC and NDHM, 21% for HC. The quantitation was performed to the limit of 5 ng/ml (signal-to-noise ratio higher than 5).The calibration linear range was fulfilled in the concentration range 0 to 3000 ng/ml with correlation coefficients better than 0.99 for DHC and DHM, better than 0.98 for NDHC and HC and 0.96 for NDHM. In case of exceeding the concentration range of linearity, the real urine samples were diluted with water as appropriate before hydrolysis and analyses were repeated.

3. Results and discussion

At present, GC–MS methods are routinely used to explore toxicological samples.

Because of a great variety of unknown toxic compounds which may be present (or not) in the sample, silylation can be a convenient derivatization reaction for molecular groups of various types and can be included in sample preparation steps before GC–MS trace analyses. From a practical point of view this attitude can be useful with respect to the fact of polydrug intoxications, economical efficiency of analyses and the restricted sample amount available in some cases.

Using MSTFA for quantitation of opiates by GC–MS we have found that this way of silylation of *N*-demethylated metabolites is rather irreproducible (C.V. 30–40%) as it was presented previously [13]. Using MSTFA with ammonium iodide [14] yielded much more reproducible results for *N*-demethylated compounds (C.V. 15%) even at low concentration values (10 ng/ml). The reproducibility of the assay of other opiates remained unaffected by the modification of silylation reagent, as well as the other parameters of validation of the assay [13].

The separation of dihydrocodeine and metabolites and their mass spectra in scan mode in a volunteer non-hydrolyzed urine sample are shown as an example in Fig. 1A and B. Careful comparison of retention and mass spectral data of analytes in samples of volunteers after dihydrocodeine application and reference substances confirmed peaks as DHC.TMS (1), DHM.2TMS (2), NDHC.2TMS (3), NDHM.3TMS (4) and HC.TMS (5). Using the GC–MS method to observe the urinary excretion of dihydrocodeine in our experimental study, traces of hydrocodone have been found in addition to the metabolites described elsewhere [5–8]. The suggested possible scheme of biotransformation of dihydrocodeine is shown in Fig. 2.

The hydrocodone presence in another non-hydrolyzed urine sample is demonstrated in more detail in Fig. 3. The concentration range of hydrocodone present in the examined urine samples of all volunteers was up to 50 ng/ml. In previous studies hydrocodone was confirmed to be a metabolite of codeine in man [9,10] but to our knowledge no evidence has been given yet that it can be a metabolite of dihydrocodeine [2,5–8]. The possible oxidation of dihydrocodeine to codeine was considered by Hufschmidt et al. [6], nevertheless it was not confirmed experimentally. However, the opposite reaction, the reduction of hydrocodone to a small extent to dihydrocodeine α and β stereoisomers, has been described by Cone [11,12]. We assume that hydrocodone can be a minor metabolite of

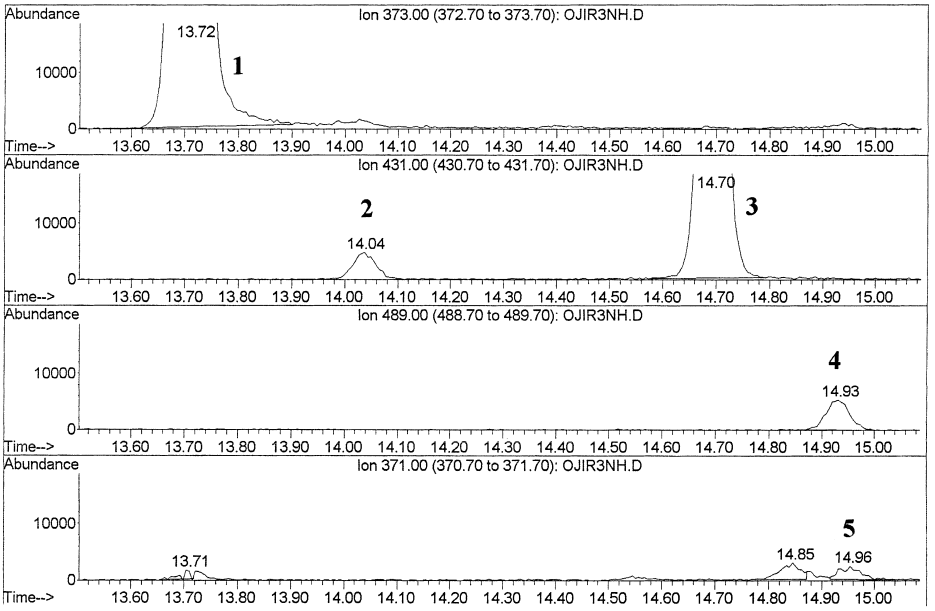

Fig. 1. (A) GC–MS scan analysis of a non-hydrolyzed human urine extract. The separation of dihydrocodeine and its metabolites in silylated forms (the appropriate mass spectra see Fig. 1B). Peaks numbered: **1** – DHC.TMS (13.72 min), **2** – DHM.2TMS (14.04 min), **3** – NDHC.2TMS (14.70 min), **4** – NDHM.3TMS (14.93 min), **5** – HC.TMS (14.96 min). (B) GC–MS scan analysis of a non-hydrolyzed human urine extract. The subtracted mass spectra corresponding to analytes the separation of which is demonstrated in Fig. 1A: Mass spectra numbered: **1** – DHC.TMS, **2** – DHM.2TMS, **3** – NDHC.2TMS, **4** – NDHM.3TMS, **5** – HC.TMS.

dihydrocodeine or the non-enzymatic oxidation product. Further studies concerning the stability of dihydrocodeine will be useful to distinguish between mechanisms of its appearance in urine.

Comparing the corresponding data resulting from analyses of hydrolyzed and non-hydrolyzed urine samples, we came to the conclusion that both dihydrocodeine, dihydromorphine and nordihydrocodeine were excreted in conjugated forms in prevailing extent. The degree of conjugation of dihydrocodeine and its *N*-demethylated metabolite, nordihydrocodeine (6-conjugates) was nearly the same, 65% on average. *O*-demethylated metabolite, dihydromorphine, excreted as 3- and 6-conjugated forms, was bound to 85%. The degree of conjugation of the minor metabolite nordihydromorphine and the possible metabolite hydrocodone has not been determined due to their low concentration in examined samples.

The relative total amounts of each compound (free and conjugated) excreted into urine during a 72 h time interval are given in Table 1. The major part of the dose administered is excreted as dihydrocodeine itself. Further, it is apparent that both dihydrocodeine and nordihydrocodeine amounts are prevailing over the other metabolites dihydromorphine and nordihydromorphine, quite in accordance with other findings [2,6,7].

4. Conclusions

With the GC–MS method used, we have indicated that hydrocodone can be a possible minor metabolite of dihydrocodeine or its non-enzymatic oxidation product. It has been found that the major part of the therapeutic dose of DHC administered to human volunteers was excreted in urine during the first 24 h interval in all individuals. Only minor remaining equivalents of the dose were eliminated via urine in the next 2 days. The analytical monitoring of DHC and metabolites in urine was possible more than 72 h after administration. The total DHC equivalent amounts excreted in urine during 72 h ranged

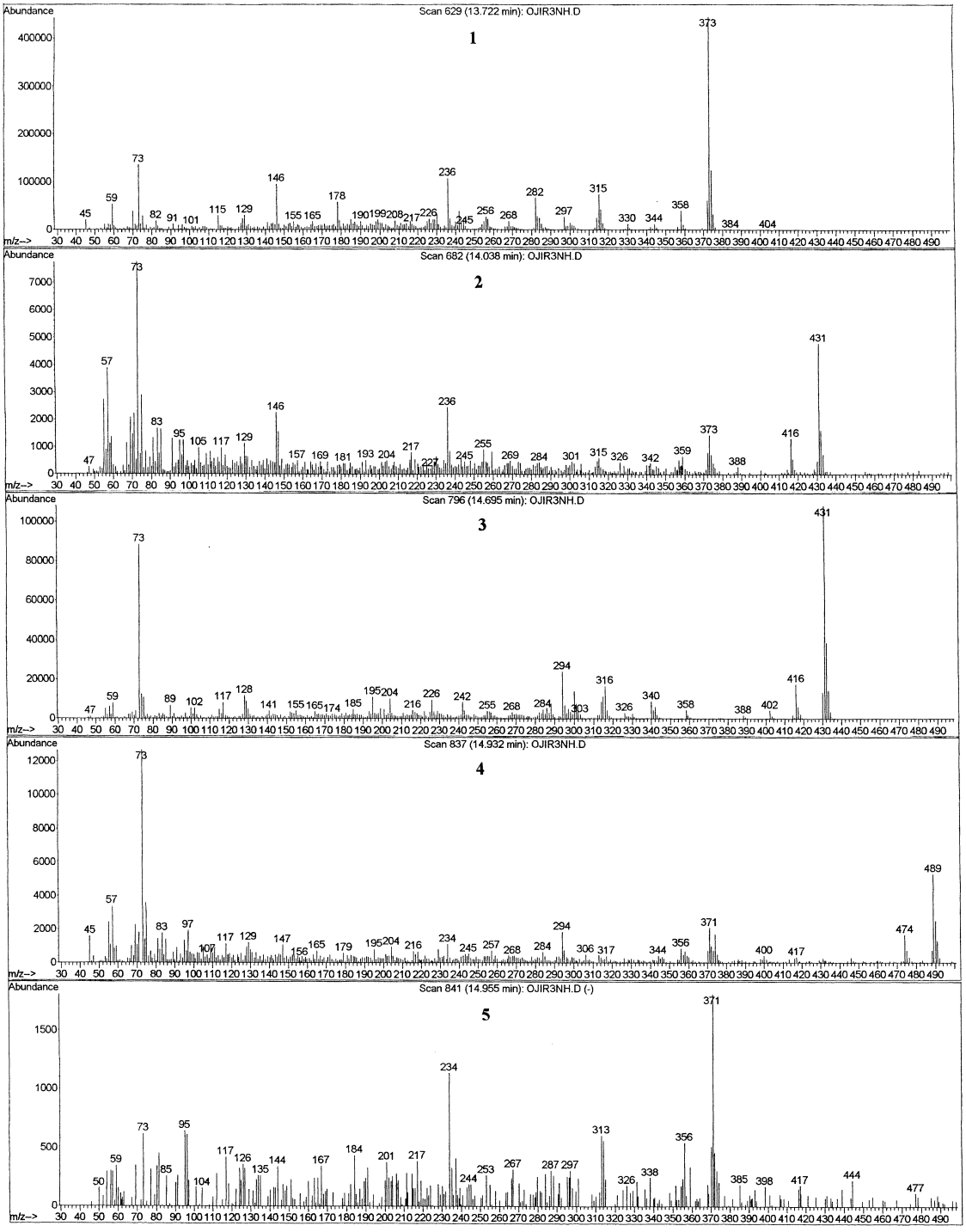

Fig. 1. (*continued*)

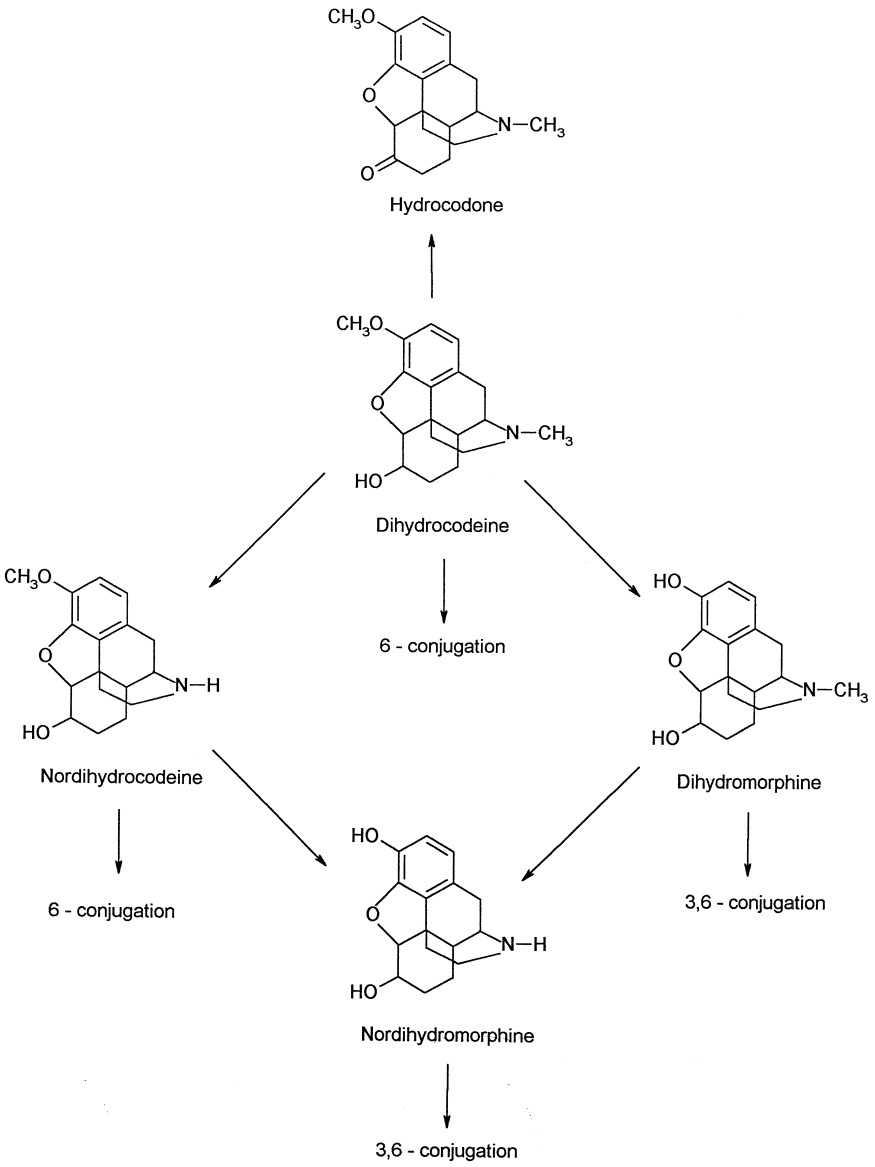

Fig. 2. Scheme of biotransformation of dihydrocodeine.

between 32 and 108% of the dose, on average 62% in all individuals in our study. On the assumption that all metabolites in urine were detected and quantified, it implies that part of the dose can be eliminated by faeces as it has been already suggested [6]. The disposition of excreted drug in urine or faeces varies strongly among individuals.

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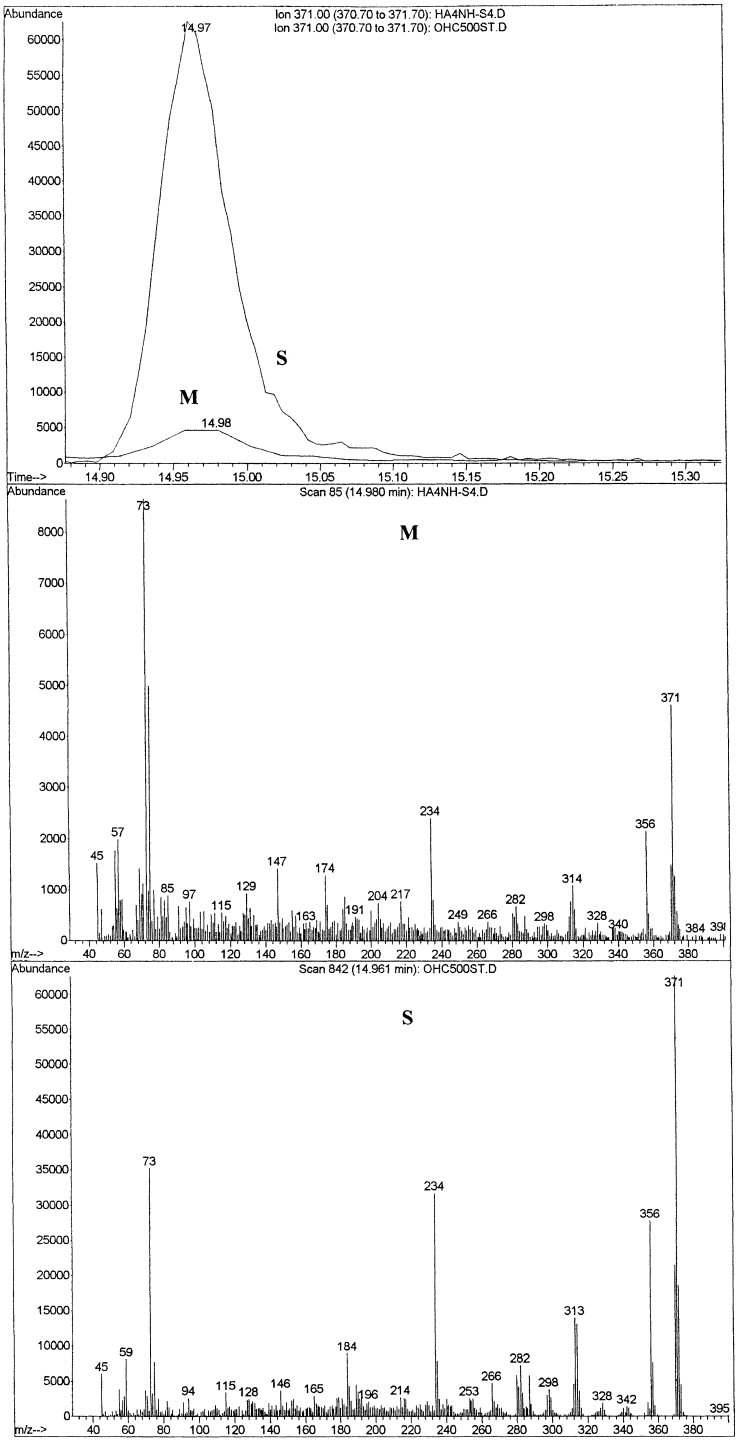

Fig. 3. Overlay of extracted ion chromatograms in detail (m/z 371, scan mode): S – Peak and mass spectrum of hydrocodone reference standard. M – Peak and mass spectrum of traces of metabolite detected in a non-hydrolyzed human urine sample.

Table 1
Total dihydrocodeine equivalents in human urine excreted after the peroral dose of tartrate salt 0.25 mg/kg

Person	Sex	Weight (kg)	Sampling interval (h)	% of Dose					
				DHC	DHM	NDHC	NDHM	HC	Sum
1	F	52	0–24	22.8	1.5	3.4	0.5	0.06	28.3
			0–48	24.6	1.8	3.9	0.8	0.1	31.2
			0–72	24.8	1.9	4.0	0.8	0.1	31.5
2	F	60	0–24	30.1	2.0	3.0	0.5	0.02	35.6
			0–48	31.4	2.3	3.4	0.9	0.03	38.1
			0–72	31.7	2.5	3.6	1.0	0.03	38.8
3	M	95	0–24	34.1	1.8	2.9	0.6	0.01	39.5
			0–48	35.6	2.0	3.3	0.8	0.01	41.9
			0–72	36.0	2.1	3.4	0.8	0.01	42.3
4	F	60	0–24	56.8	4.0	7.0	1.3	0.2	69.3
			0–48	57.9	4.5	7.5	1.6	0.2	71.7
			0–72	58.0	4.7	7.6	1.7	0.2	72.1
5	F	63	0–24	63.7	2.9	8.0	1.3	0.2	76.1
			0–48	65.9	3.3	8.3	1.5	0.2	79.1
			0–72	66.9	3.3	8.3	1.6	0.2	80.3
6	F	57	0–24	76.5	7.1	15.1	3.8	0.4	102.4
			0–48	77.8	7.7	16.0	4.8	0.5	106.8
			0–72	78.0	7.9	16.2	5.0	0.6	107.6

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