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Urinary concentrations of codeine and morphine after the administration of different codeine preparations in relation to doping analysis*

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Abstract: A capillary GC method with nitrogen-specific detection is described for the analysis of codeine and morphine in urine. Both drugs were determined after enzymatic hydrolysis of the urine. Morphine was derivatized with trifluoroacetic anhydride. For 5-ml samples of urine, the lower detection limits for accurate quantitation were 50 ng ml^{-1} and 100 ng ml^{-1} for morphine and codeine, respectively. Both codeine and morphine were already detectable in urine 1 h after the intake of the analgesic preparation Perdolan®. Codeine excretion and concentration peaked 2 h after administration of a dose. The percentage of the dose excreted as codeine was 3.0–6.2%. Administration of the antitussive preparation Bisolvon Griblettes® resulted in detectable codeine and morphine levels for at least one day; 5.6–9% was excreted as total codeine over 24 h, the conjugated metabolite morphine accounting for 1.7–7.4% of the dose. Nearly the same amounts of codeine and morphine were excreted after administration of the antitussive syrup Bronchodine®. The maximum excretion rate of codeine occurred after 1 h. Generally codeine and morphine remained detectable for 12 h. The results of these administration studies are discussed in relation to the codeine and morphine threshold levels recently introduced by the International Cyclist Union.

Keywords: *Codeine; morphine; doping analysis.*

Introduction

Codeine is a constituent of many non-prescription drug preparations including cold remedies and analgesic combinations. The natural alkaloid codeine and the semi-synthetic derivatives dihydrocodeine and oxycodone are used as antitussive drugs but can also produce dependence. Although the abuse liability of codeine is lower than that of morphine, codeine was classified as a banned drug by the Medical Commission of the International Olympic Committee. The presence of codeine, irrespective of its concentration in urine, automatically resulted in a positive doping test. Codeine is also mentioned on the list of doping agents published in 1987 by the Ministry of Health of the Flemish Executive. However the doping test should be regarded as positive if 'abnormally' high quantities of codeine are found in the urine. The objectives of the present study were to determine: the urinary concentration range of codeine that might be encountered after the administration of therapeutic amounts of several preparations containing codeine; and if a threshold level of codeine could be considered for doping control purposes. At the end of this study, however, a decision was made by the Medical Commission of the International Cyclist Union (UCI) to allow urinary concentrations of codeine and morphine (as a metabolite of codeine) below 1 μg ml^{-1} [1]. Therefore, after the determination of codeine the same urine samples were re-analysed by GC in order to quantitate morphine.

Although GC separation of codeine can be accomplished directly [2, 3], the phenolic hydroxy group in morphine must be derivatized before GC separation and detection. Various derivatization procedures including acetylation [4, 5], silylation [6] and trifluoroacetylation have been used [7]. Recently, five common derivatization methods for morphine and codeine were evaluated in order to compare the accuracy and precision of a quantitative GC–MS procedure [7]. In the present work codeine will be detected by capillary GC with nitrogen specific detection. For the GC quantitation of morphine the extraction and derivatization procedure of Wallace *et al.* [8] was modified.

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Materials and Methods

Experimental design and subjects

The study was performed on four healthy volunteers. The nature and purpose of the study was explained to each volunteer before asking for their consent to participate. The volunteers were asked not to take any medication for 1 week before the experiment. The following preparations were consecutively administered with a period of 2 weeks between each preparation: two tablets of the antitussive tablets Bisolvon Griblettes® (Boehringer, Ingelheim, Germany), each containing acetylsalicylic acid (300 mg), bromhexine hydrochloride (4 mg) and codeine phosphate (15 mg); 15 ml of the antitussive syrup Bronchodine® (Pharmethic, Brussels) containing 30 mg codeine phosphate; and one tablet of the analgesic tablets Perdolan® (Janssen Pharmaceutica, Beerse, Belgium) containing acetylsalicylic acid (200 mg), bromisoval (10 mg), carbromal (30 mg), caffeine (50 mg), paracetamol (200 mg) and codeine phosphate (10 mg).

Urine was collected in capped bottles before (0 h) and 1, 2, 3, 4, 6, 9, 12, 24 and 30 h after administration of codeine and was either analysed immediately or stored deep-frozen for later analysis. Urinary pH and volume were measured and all samples were analysed in duplicate.

Reagents and apparatus

Codeine base and mepivacaine hydrochloride were obtained from Sigma Chemie (Deisenhofen, Germany) and Astra (Södertälje, Sweden), respectively. Morphine and ethylmorphine were obtained from Bios (Brussels).

Ammonia buffer (pH 9.5) was prepared by the addition of ammonia to a saturated ammonium chloride solution. Sodium acetate and acetic acid were used for the preparation of 1 M acetate buffer (pH 5.2). *Helix pomatia* juice (SHP), containing β-glucuronidase 100,000 Fishman units ml^{-1} and sulphatase 1000,000 Roy units ml^{-1}, was obtained from IBF (Villeneuve, France). Trifluoroacetic anhydride (TFAA) was obtained from Pierce (Oud-Beijerland, The Netherlands).

All chromatograms were generated in the split mode (split ratio 1:10) on a Varian (Walnut Creek, CA, USA) 3400 gas chromatograph fitted with a 25 m × 0.22 mm i.d. fused silica CP Sil 5 CB column (Chrompack, Antwerpen, Belgium) with a film thickness of 0.11 μm. The GC was equipped with an autosampler and a nitrogen-specific detector and was interfaced with an IBDH data processor. Injector and detector temperatures were maintained at 280°C. The oven temperature was programmed as follows: initial temperature 160°C; initial hold 1 min; temperature program rate 10°C min^{-1}; and final temperature 280°C. Helium was used as the carrier gas at an inlet pressure of 1.1 bar. Detector make-up flow rate was 25 ml min^{-1}.

Methods of determination of codeine and morphine

Codeine. A 5 ml volume of urine in a screw-capped tube was buffered with 1 ml of 1 M sodium acetate buffer (pH 5.2) and 50 μl of SHP were added. The urine was hydrolysed for 2 h at 56°C. After cooling, the hydrolysate was made alkaline by adding 0.5 ml of ammonia buffer followed by 50 μl of internal standard solution (mepivacaine HCl, 50 μg ml^{-1} in water) and was extracted by rolling with 5 ml of CH_2Cl_2:MeOH (9:1, v/v) for 15 min. After centrifugation the organic phase was transferred to a screw-capped tube and evaporated under nitrogen at 50°C. The residue was redissolved in 1 ml of 0.1 M HCl and mixed in a vortex mixer with 2 ml of diethyl ether for 30 s. The organic phase was discarded and the aqueous solution was made alkaline by adding 0.5 ml of ammonia buffer. Extraction with 5 ml of CH_2Cl_2:MeOH (9:1, v/v) was performed by rolling for 15 min. After centrifugation (5 min) the organic layer was separated, dried over anhydrous Na_2SO_4 and evaporated under nitrogen at 50°C. The residue was dissolved in 150 μl of ethyl acetate–methanol (9:1, v/v) and transferred to an appropriate autosampler microvial (200 μl); 1 μl was injected on the chromatograph. Codeine was also measured in unhydrolysed urine.

A standard curve was obtained by subjecting spiked urine samples (0, 0.05, 0.10, 0.25, 0.50, 1 and 2 μg ml^{-1}) to the above extraction procedure in quadruplicate for each concentration. The accuracy of the assay was measured for two different codeine concentrations (0.25 and 1 μg ml^{-1}).

Morphine. With the same procedure as for codeine, 5 ml of urine was hydrolysed; the

hydrolysate was cooled, made alkaline by adding 0.5 ml of ammonia buffer followed by 50 μl of the internal standard (ethylmorphine 50 μg ml^{-1} in methanol) and extracted as for codeine. The residue was redissolved in 50 μl of ethyl acetate and 50 μl of TFAA was added. Derivatization was performed at 60°C for 30 min. The excess reagent was then removed by evaporation under nitrogen at 60°C and the residue was dissolved in 200 μl of ethyl acetate and transferred to an autosampler microvial (200 μl); 1 μl was injected on the chromatograph. A standard curve was constructed by analysing spiked urine samples (final concentrations 0.05, 0.1, 0.25, 0.50 and 1 μg ml^{-1}) in quadruplicate for each concentration.

The stability of the TFA derivatives was evaluated by separately derivatizing 50 μg of morphine and 50 μg of internal standard with 200 μl of TFAA. After evaporation under nitrogen the residues were dissolved in 2 ml of ethyl acetate and divided among several autosampler vials. These vials were then analysed after 0.30 min and 1, 2, 3, 6 and 24 h, respectively.

Results and Discussion

Determination

Under the chromatographic conditions described codeine and the internal standard gave sharp peaks with retention times of 8.02 and 5.55 min, respectively.

From the chromatograms obtained after the extraction of spiked urine a standard curve was generated by plotting the codeine: mepivacaine peak area ratio against the codeine concentration. The statistical data for the slope, intercept and the correlation coefficient were 0.937, 0.026 and 0.9988, respectively. For 5-ml samples of urine, the lower limit for accurate quantitative detection of codeine was 100 ng ml^{-1} (signal-to-noise ratio = 4). The accuracy and precision of the assay are summarized in Table 1.

Table 1
Accuracy and precision of the codeine assay

Sample (n =4)	Concentration (μg ml^{-1}) added	found	RSD* (%)
Day 1	1	1.022 ± 0.053	5.2
	0.25	0.245 ± 0.011	4.5
Day 2	1	1.011 ± 0.054	5.3
	0.25	0.240 ± 0.016	6.6

*RSD = Relative standard deviation.

Derivatization of morphine and the internal standard ethylmorphine resulted in symmetrical GC peaks with retention times of 11.9 and 12.8 min, respectively. Under these conditions trifluoroacetylated codeine was eluted after 12.3 min. The calibration curve showed good linearity between peak–area ratios and concentrations of 0–1000 ng ml^{-1} ($y = 0.286x - 0.0025$, $r = 0.9982$). The lower limit for accurate quantitative determination of morphine was 50 ng ml^{-1}.

Several procedures for trifluoroacetylation of morphine are known [5, 8, 9] of which trifluoroacetylation at 60°C for 30 min [9] was found to be most suitable. Paul *et al.* [5], however, observed breakdown of the di-TFA derivative to the mono-TFA product and eventually to the parent compound after 4 h. Analogous difficulties due to the poor stability of 3,6 di-TFA-morphine were encountered in the comparative derivatization study of Chen *et al.* [7]. Moreover, their choice of an internal standard (nalorphine) was not appropriate as apparently incomplete trifluoroacetylation of this drug resulted in split GC-peaks. The results of the stability test in the present work, however, indicated that even after 24 h no breakdown of the TFA derivatives of morphine and ethylmorphine occurred. It should be noted, however, that great care was taken to dry the urine extracts before the derivatization step. To avoid hydrolysis during the GC analysis overnight, the microvials were completely filled with the redissolved residue and properly sealed. Indeed, it is well known that perfluoroesters are unstable in the presence of moisture [10, 11] owing to the strong negative inductive effects of the fluorine atoms. The handling of these derivatives therefore requires anhydrous conditions.

Urinary excretion

The urinary excretion rate and concentration of total codeine and morphine after the intake of Perdolan® by the four subjects is given in Table 2. From these results it appeared as soon as 1 h after the intake of the preparation both codeine and morphine could be detected in urine. Codeine remained detectable until 6–9 h whereas morphine was still present in the urine after 12 h. On the whole, codeine excretion and concentration peaked 2 h after the dose had been given. Codeine con-

Table 2
Excretion rate (μg h^{-1}) and concentration (μg ml^{-1}) of codeine and morphine after the intake of the analgesic preparation Perdolan® by four subjects

Time (h)	Codeine								Morphine							
	Excretion rate				Concentration				Excretion rate				Concentration			
	I	II	II	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
1	83	37	110	91	1.3*	0.7	0.2	2.1*	27	6	33	13	0.4	0.1	0.1	0.3
2	110	57	66	94	2.0*	0.9	0.6	4.7*	49	14	22	13	0.9	0.2	0.2	0.6
3	91	30	43	†	0.5	0.4	0.5	†	57	11	14	†	0.3	0.2	0.1	†
4	14	49	34	71	0.2	0.7	0.5	2.1*	29	12	8	14	0.4	0.2	0.1	0.4
6	12	27	27	25	0.1	0.1	0.3	0.4	26	30	8	21	0.2	0.1	0.1	0.3
9			14	17			0.2	0.3	22	—	5	15	0.1	—	0.1	0.7
12				12				0.2	14	9	4	15	0.1	0.1	0.1	0.2
24									11	—		9	0.1	—		0.1
30									16	—			0.1	—		
36										10			—	0.1		

*UCI positive.
†No urine collection.

centrations above 5 μg ml^{-1} were not found; the mean codeine concentration in this experiment was 0.86 ± 1.06 μg ml^{-1}. A mean maximum morphine concentration of 0.37 ± 0.2 μg ml^{-1} was generally obtained 2 h after the intake of Perdolan®.

The percentage of the dose excreted as total codeine was low (3.0–6.2%). Unconjugated codeine accounted for 1.93–5.73% whereas conjugated morphine represented 1.8–6.3% of the total dose. The metabolic ratio for *O*-demethylation (codeine:morphine) after 24 h varied from 1.5 to 2.9 except for subject I where more morphine than codeine was eliminated renally.

Based on the recent UCI regulations, the intake of one tablet of Perdolan® will result in a positive doping test for codeine in subjects I and IV during periods of 2 and 4 h after dosing, respectively. UCI positive morphine concentrations were not found in any of the subjects.

As shown in Table 3 the greater amount of codeine in Bisolvon Griblettes® compared to Perdolan®, resulted in higher urinary codeine concentrations in all subjects. Generally codeine was detectable from 1 to 24 h with a maximum excretion rate after 2 h (except for subject I). The mean codeine concentration of this experiment was 3.1 ± 3.5 μg ml^{-1}, the highest value being 13.3 μg ml^{-1} in subject II.

The percentage of the dose excreted as total codeine over 24 h varied from 5.6 to 9.0%; unconjugated codeine represented 3.9–6.7% whereas the conjugated metabolite morphine accounted for 1.7–7.4% (mean 4.0 ± 1.9%) of the total dose. Inter-individual variation was found in the morphine peak concentration time; the maximum value was found in subject II after 2 h (6.2 μg ml^{-1}). The metabolic ratio for *O*-demethylation was 1.1–2.4.

The intake of this antitussive codeine preparation in therapeutic amounts would result in a UCI positive doping test for all subjects during 4 h. For three of them the test remained positive even 6 h after administration.

The faster absorption and higher bioavailability of codeine after the administration of the syrup Bronchodine® could account for the maximum codeine excretion rate occurring as soon as 1 h after dosing. The percentage of the dose excreted as total codeine was slightly higher than after Bisolvon® (same amount of codeine) and varied from 6.0–11.3% (24 h) with conjugated codeine ranging from 3.7 to 7%. The mean codeine concentration in the urine of the four subjects during the whole experiment was 2.8 ± 3.0 μg ml^{-1}; the highest value was found in Subject IV after 2 h (11.8 μg ml^{-1}). Noticeable differences in the four subjects occurred both in morphine excretion rate and concentration. The percentage of the dose excreted as morphine after 24 h ranged from 1.6–6.7% (subject II not included).

Compared to the other experiments, relatively more codeine than morphine was excreted after the intake of Bronchodine®. The metabolic ratio for *O*-demethylation was 1, 4 and 9 for subjects I, II and IV, respectively. The therapeutic use of this antitussive syrup would result in UCI positive doping tests for all

Table 3
Excretion rate ($\mu g\ h^{-1}$) and concentration ($\mu g\ ml^{-1}$) of codeine and morphine after the intake of the Bisolvon Griblettes® by four subjects

Time (h)	Codeine								Morphine							
	Excretion rate				Concentration				Excretion rate				Concentration			
	I	II	II	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
1	512	74	308	265	5.1*	1.9*	0.8	4.6*	262	32	59	30	2.6*	0.8	0.2	0.5
2	412	345	309	464	1.7*	13.3*	2.7*	9.3*	201	162	51	58	0.8	6.2*	0.4	1.2*
3	229	257	211	360	2.4*	10.7*	6.4*	8.8*	70	122	29	58	0.7	5.1*	0.9	1.4*
4	143	121	122	184	1.4*	5.5*	2.5*	5.3*	80	65	16	36	0.8	3.0*	0.3	1.0*
6	38	70	57	156	0.5	2.3*	1.1*	3.6*	77	62	12	36	0.9	2.0*	0.2	0.8
9	31	47	45	48	0.2	1.5	0.9	0.8	42	35	12	55	0.3	1.1*	0.2	0.9
12	18	25	24	43	0.1	0.4	0.4	0.7	40	27	11	27	0.3	0.4	0.2	0.5
24		10	10	14		0.1	0.2	0.2	18	33	6	24	0.3	0.3	0.1	0.4
30									28	13	7		0.2	0.3	0.1	
36									31				0.3			

*UCI positive.

Table 4
Excretion rate ($\mu g\ h^{-1}$) and urinary concentration ($\mu g\ ml^{-1}$) of codeine and morphine after the intake of Bronchodine® by four subjects

Time (h)	Codeine								Morphine							
	Excretion rate				Concentration				Excretion rate				Concentration			
	I	II	II	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
1	459	384	404	560	2.1*	7.5*	3.3*	8.2*	200	24	65	53	0.9	0.5	0.5	0.8
2	357	302	382	354	1.1*	7.9*	2.1*	11.8*	248	35	72	43	0.8	0.9	0.4	1.4*
3	218	204	126	248	1.9*	3.5*	2.1*	7.1*	119	†	41	41	1.1*	†	0.7	1.2*
4	192	142	138	351	0.6	4.3*	0.9	5.4*	137	17	48	62	0.5	0.5	0.3	1.0*
6	57	77	53	179	0.7	3.3*	1.3*	3.9*	68	†	18	32	0.8	†	0.5	0.7
9	39	34	53	112	0.4	1.1*	1.0*	1.6*	51	5	15	26	0.6	0.2	0.3	0.4
12	22	19	19	61	0.1	0.3	0.2	0.7	53		11	30	0.3		0.2	0.4
24			6	15			0.1	0.2	23			12	0.3			0.2
30									18				0.2			
36																

*UCI positive.
†Amount of urine not sufficient to determine both codeine and morphine.

subjects for at least 3 h and for three of them even for 9 h after dosing.

Codeine and morphine threshold levels

Several authorities including the International Olympic Committee (IOC), the International Cyclist Federation (UCI) and many national doping committees have two different approaches to the misuse of drugs in competitive sports. Indeed there is a complete ban for compounds such as amphetamines, exogenous anabolic steroids, beta-blocking agents and diuretics. On the other hand, threshold levels were introduced for the social drug caffeine and the endogenous steroid testosterone.

A third class was created by a recent decision of the Medical Commission of the UCI allowing morphine and codeine below 1 $\mu g\ ml^{-1}$. However, the introduction of permitted levels for these drugs implies admitting and consenting to their therapeutic use and more specifically, the use of codeine as an antitussive. The oral codeine dose for suppression of cough is 10–20 mg [12]. As illustrated in Tables 2–4 the application of the UCI codeine threshold level after the administration of the therapeutic dose still resulted in 83 and 58% positive tests, respectively, 2 and 4 h after administration. It is concluded that the proposed permitted value of 1 $\mu g\ ml^{-1}$ for codeine is much too low.

Formerly codeine was a banned drug since in endurance sports it could be abused, especially for its analgesic effect. However some side-effects of the opioids including drowsiness, respiratory depression, nausea and vomiting are clearly detrimental to athletic performance. On the other hand codeine has an exceptionally low affinity for opioid receptors and the analgesic effect of codeine is due to its

conversion to morphine. Hence a threshold level for morphine would be more appropriate in order to detect the abuse of both codeine and morphine. The mean maximum morphine concentration for the three experiments is 1.4 ± 1.6 μg ml^{-1}. A 95% confidence interval would result in a morphine threshold level of 4.6 μg ml^{-1}. Moreover, based on the maximum values after Bisolvon Griblettes® (2.8 ± 2.4 μg ml^{-1}), a much higher permitted level (95% confidence interval) of 7.6 μg ml^{-1} is needed. Notwithstanding the relatively small number of subjects these results indicate that the UCI morphine level of 1 μg ml^{-1} is also too low. Furthermore a 160-fold inter-individual variation in metabolic rate for *O*-demethylation was found in Caucasians while inter-ethnic differences in codeine excretion and metabolism were also noticed [13]. More experiments with Bisolvon Griblettes® are therefore needed in order to establish a reliable morphine threshold value.

Alternatively, since several non-banned analgesics and different non-narcotic antitussive preparations are available, a complete ban of codeine and morphine seems to be more appropriate and more adequate than the current UCI regulation which provides little control on athletes. If, however, the permitted threshold of 1 μg ml^{-1} for morphine and codeine is retained, all athletes should be warned against taking preparations containing codeine at least 24 h before an event.

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