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[Article]

# Determination of Urinary Metabolites of Caffeine for the Assessment of Cytochrome P4501A2, Xanthine Oxidase, and *N*-Acetyltransferase Activity in Humans

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## Abstract

Summary: Caffeine metabolism via the 3-demethylation pathway is sequentially catalyzed by cytochrome P4501A2 (CYP1A2), xanthine oxidase, and *N*-acetyltransferase. The activities of the three enzymes can be estimated from urinary metabolic ratios of four caffeine metabolites, 5-acetylamino-6-formylamino-3-methyluracil (AFMU), 1-methyluric acid (1MU), 1-methylxanthine (1MX), and 1, 7-dimethyluric acid (17DMU), after the ingestion of caffeine. A method for quantitation of the four metabolites in human urine has been developed. The method is based on a one-step extraction with ethyl acetate/2-propanol followed by high-performance liquid chromatography with UV detection. The detection limit was 1 µ*M* for AFMU, 1MU, and 1MX and 2 µ*M* for 17DMU. The intraday and interday coefficients of variation were <3% and <7%, respectively, and the accuracy was within ±3%. The method was employed in a population study of 277 healthy volunteers, each of whom ingested 200 mg caffeine and provided a urine sample [almost equal to]6 h later. The metabolite concentration ranges in the urines were 2.1-327 µ*M*, 4.0-744 µ*M*, 4.9-598 µ*M*, and 6.4-260 µ*M* for AFMU, 1MU, 1MX, and 17DMU, respectively. The CYP1A2 ratio (AFMU + 1MU + 1MX/17DMU) was significantly lower in women than in men, excluding smokers and oral contraceptive users. The CYP1A2 ratio was higher in smokers than in nonsmokers, confirming the induction of CYP1A2 by smoking. In women using oral contraceptives, the CYP1A2 ratio was, as expected, significantly lower than in women not using oral contraceptives. For the *N*-acetyltransferase ratio (AFMU/1MX) and the xanthine oxidase ratio (1MU/1MX), no differences were seen in terms of sex, smoking habits, or the use of oral contraceptives. All results are in agreement with previous reports on CYP1A2, *N*-acetyltransferase, and xanthine oxidase activities in humans. Thus, the method is both analytically and biologically reliable for the assessment of CYP1A2, *N*-acetyltransferase, and xanthine oxidase in humans.

The cytochrome P4501A2 enzyme (CYP1A2) is one of the major cytochrome P450s in the liver, accounting for [almost equal to]15% of the total P450 content (1). Until now attention has mainly been drawn toward the role of CYP1A2 as an activator of procarcinogens (2-4). However, CYP1A2 is also a catalyst of the biotransformation of many clinically used drugs, such as theophylline and caffeine (2,5), clozapine (6-8), propranolol (9,10), imipramine (11) and tacrine (12). Thus, there is an interest in assessing the activity of CYP1A2 in patients before treatment with CYP1A2 substrates, in particular those that have a low therapeutic index and for which clinical dose titration is not feasible, e.g., theophylline, clozapine, and imipramine.

As an innocuous drug, caffeine is still the model of choice for assessing CYP1A2 activity. Caffeine (1,3,7-trimethylxanthine, 137TMX) is predominantly eliminated via 3-demethylation to paraxanthine (1,7-dimethylxanthine, 17DMX), catalyzed by CYP1A2 (2,13,14). Via an unknown intermediate, 17DMX is converted to 5-acetylamino-6-formylamino-3-methyluracil (AFMU) by *N*-acetyltransferase (15,16). In addition, 17DMX is hydroxylated to 1,7-dimethyluric acid (17DMU) and demethylated by CYP1A2 to 1-methylxanthine (1MX); the latter is further hydroxylated to 1-methyluric acid (1MU) by xanthine oxidase (17) (Fig. 1). Thus, the activities of CYP1A2, *N*-acetyltransferase, and xanthine oxidase can be estimated from the ratios of the metabolites excreted into the urine after the ingestion of a single dose of caffeine (18,19).

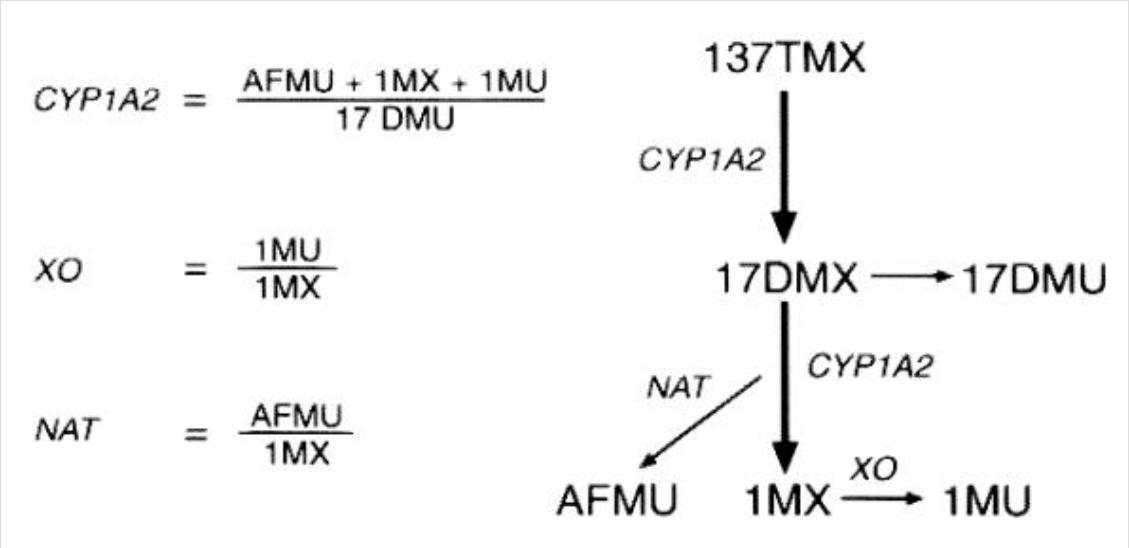


**FIG. 1.** The 3-demethylation pathway of caffeine (137TMX) and the urinary metabolic ratios reflecting CYP1A2, xanthine oxidase (XO), and *N*-acetyltransferase (NAT) activities in humans. AFMU, 5-acetylamino-6-formylamino-3-methyluracil; 1MU, 1-methyluric acid; 1MX, 1-methylxanthine; 17DMU, 1,7-dimethyluric acid.

A number of urinary caffeine metabolite ratios have been proposed as a measure of CYP1A2 activity: AFMU + 1MX + 1MU/17DMU (18), 17DMX + 17DMU/137TMX (20), 17DMX/137TMX (21), and AFMU + 1MX + 1MU + 17DMX + 17DMU/137TMX (22). The last three ratios reflect the 3-demethylation of caffeine to 17DMX, which is almost exclusively catalyzed by CYP1A2 (2,13,14). The ratio AFMU + 1MX + 1MU/17DMU proposed by Campbell et al. (18) reflects the 7-demethylation of 17DMX, which is also catalyzed by CYP1A2 (5,23), although it does not seem to be as highly dependent on CYP1A2 as the 3-demethylation pathway of caffeine (24). However, taking into account that the urinary elimination of both 17DMU and 137TMX is dependent on urine flow (25) and that 137TMX excretion into the urine is very small, there is great risk of analytical error. Hence the AFMU + 1MU + 1MX/17DMU ratio, in our opinion, is the analytically most feasible and thus was used in our study.

Caffeine metabolism exhibits dose dependency (26), and in order to avoid this confounding effect we have abandoned the intake of methylxanthines 40 h before caffeine intake and standardized the dose to a single one of 200 mg caffeine. Furthermore, it has previously been shown that the CYP1A2 ratio measured in urine collected 5-8 h after caffeine intake correlates highly with caffeine 3-demethylation clearance (27). In our study we have standardized the urine collection time to a sample collected 6 h after caffeine intake.

The main purpose of this study was to develop a high-performance liquid chromatography (HPLC) method for the determination of the caffeine metabolites AFMU, 1MU, 1MX, and 17DMU in urine for the assessment of CYP1A2, *N*-acetyltransferase, and xanthine oxidase activities in humans. In order to improve working safety in the laboratory, the aim was to substitute the highly toxic solvent chloroform, which is commonly used for extraction of methylxanthines from human urine (18-20), with a less hazardous solvent. Here we describe a method based on a simple one-step extraction procedure with ethyl acetate/2-propanol as the extraction solvent. The method has been validated analytically and biologically by means of a population study.

## MATERIALS AND METHODS

### Protocol

The study was approved by the regional ethics committee of the counties of Vejle and Funen. Two hundred seventy-seven healthy Danish volunteers, 143 men and 134 women, were included in the study after giving informed written and verbal consent. The median age was 22 years (range 18-43 years). forty-one subjects stated that they were smokers (range 1-30 cigarettes per day), and 64 female subjects used oral contraceptives. The subjects abstained from ingesting methylxanthine-containing foods, beverages, and medication for >=40 h before the study and until delivery of the urine sample.

Each subject ingested 200 mg caffeine (Nycomed DAK, Copenhagen, Denmark). Approximately 6 h later each one provided a urine sample in a conical tube containing 300 µl 1 N HCl. The urine sample was stored at -20°C until analysis. At the same time, all subjects were phenotyped according to sparteine (CYP2D6) and mephenytoin (CYP2C19) oxidation polymorphisms by combined administration of 100 mg mephenytoin (Mesantoin; Sandoz Pharmaceuticals Corp., East Hanover, NJ, U.S.A.) and 100 mg sparteine sulfate (Depasan; Giulini Pharma GmbH, Hannover, Germany). *S*- and *R*-mephenytoin in urine were assayed by a modification of a previously published gas chromatographic method (28).

In order to avoid use of the toxic solvent chloroform, ethyl acetate was used instead for the extraction of the two compounds from urine. The mephenytoin *S/R* ratio was calculated as the ratio between the peak areas of the *S*- and *R*-enantiomers in a 15-h urine sample (28,29). A mephenytoin *S/R* ratio >=0.9 defined the poor metabolizers of mephenytoin, and an *S/R* ratio <0.9 defined the extensive metabolizer phenotype of mephenytoin. In the same urine sample, sparteine and dihydrosparteine were assayed by a modification of a previously published gas chromatographic method (30); instead of the toxic solvent dichloromethane, *tert*-butylmethylether was used for the extraction of sparteine and metabolites from urine. The sparteine metabolic ratio (MR) was determined as the ratio between excreted sparteine and 2,3- plus 5,6-dehydrosparteine (30). A sparteine metabolic ratio >=20 defined the poor metabolizers of sparteine, and a MR <20 defined the extensive metabolizers of sparteine.

### Analysis of Caffeine Metabolites

#### Chemicals and Reagents

1MX, 17DMU, and [beta]-hydroxy-ethyl theophylline ([beta]-OH-ET) were purchased from Sigma (St. Louis, MO, U.S.A.), and 1MU was purchased from Fluka AG (Buchs, Switzerland). AFMU was kindly donated by Mr. R. Fumeaux, Nestlé (Genève, Switzerland), and ethyl acetate, 2-propanol, ethanol, and -methanol (HPLC grade) were obtained from Merck (Darmstadt, Germany). All other chemicals were of analytical grade and obtained from either Merck or Sigma. Water was purified by osmosis and distillation.

#### Preparation of Standard Solutions

Stock solutions of 5 m*M* 1MU, 1MX, 17DMU, and the internal standard, [beta]-OH-ET, were prepared in 30% vol/vol ethanol in distilled water. AFMU (5 m*M*) was prepared in acetate buffer (0.01 *M*, pH 4.0). From the stock solutions, a standard mixture (400 µ*M*) of 1MU, 1MX, and 17DMU was prepared in distilled water, and a standard solution (400 µ*M*) of AFMU was prepared in acetate buffer, pH 4.0. The mixtures were used to prepare the appropriate standards in urine collected from a subject after 4 days of xanthine-free diet.

#### Extraction Procedure

During preparation and extraction and after reconstitution, all samples were kept on ice in order to conserve AFMU. To a 40-µl sample of urine, 100 µl of 160 µ*M* internal standard solution was added in a 10-ml glass test tube, and the total volume was adjusted to 400 µl with acetate buffer (0.01 *M*, pH 4.0). Subsequently, 5 ml ethyl acetate/2-propanol (93:7 wt/wt) was added. The mixture was shaken for 10 min, and after centrifugation for 10 min at 1,000 *g* the tube was maintained at -30°C until the aqueous phase was frozen. The organic phase was transferred to a conical glass test tube and evaporated to dryness at 55°C under a steam of nitrogen. The residue was reconstituted in 300 µl mobile phase, vortex-mixed for 10 s, and centrifuged for 30 s at 1,000 *g*. A 40-µl aliquot was injected into the column.

#### Apparatus and Chromatographic Conditions

Chromatography was performed using Hitachi instruments (Hitachi, Tokyo, Japan): an AS-2000 autosampler with a 100-µl injector loop, a T-6300 column thermostat, an L-6200 intelligent pump, and an L-4250 UV-VIS detector with variable wavelength. The system was controlled through a D-6000 HPLC interface module and a personal computer (IBM). In the autosampler the samples were kept at 5°C by a Heto CB 8-30c water bath controlled by a DT Hetotherm thermostat. Separations were achieved using a Beckman Ultrasphere ODS column (5 µm, 250 × 4.6 mm internal diameter) (Beckman Instruments Inc., Fullerton, CA, U.S.A.). The mobile phase consisted of acetate buffer (0.01 *M*, pH 4.0) and methanol (91:9 wt/wt). The aqueous phase was filtered through a Millipore filter (0.45 µm), and the mobile phase was degassed before use. The column temperature was 30°C, and the flow rate was kept at 1 ml/min from 0 to 11 min, at 1.5 ml/min from 11 to 17 min, and at 2.5 ml/min from 17 to 30 min. The column effluent was quantified at a wavelength of 273 nm.

#### Statistics

The metabolic ratios of caffeine were calculated according to the formulas given in Fig. 1. Thus, CYP1A2 activity was estimated from the ratio AFMU + 1MU + 1MX/17DMU, *N*-acetyltransferase activity from the ratio AFMU/1MX, and xanthine oxidase activity from the ratio 1MU/1MX. The distribution of the three metabolic ratios of caffeine was investigated by judging the shape of frequency distributions and probit plots. The subjects were divided into groups according to gender, smoking habits, and the use of oral contraceptives, and the metabolic ratios between the groups were compared by means of median differences and 95% confidence intervals. A 95% confidence interval for the median difference that did not include zero was considered statistically significant. The caffeine metabolic ratios were compared with the sparteine metabolic ratio and the mephenytoin *S/R* ratio by the Spearman's rank correlation test. A p value $<0.05$ was considered statistically significant. Statistical analysis was carried out by use of the MEDSTAT program package, version 2.1 (1988; Astra Group, Albertslund, Denmark).

## RESULTS

### Analytical Validation

#### Selectivity

Baseline separation was obtained between the four metabolites of interest, the internal standard, and all other known caffeine metabolites under the applied conditions (Fig. 2b,c). About 30 min were required for the analysis, to ensure that all caffeine metabolites and caffeine itself had passed through the HPLC system. The retention times were 3.5, 5.8, 7.6, 13.9, and 19.8 min for AFMU, 1MU, 1MX, 17DMU and [beta]-OH-ET, respectively. Some impurities were seen in the blank chromatogram (Fig. 2a); they are possibly xanthines and uric acids originating from the breakdown of endogenous compounds. A typical chromatogram after extraction from urine from a volunteer is illustrated in Fig. 2d.

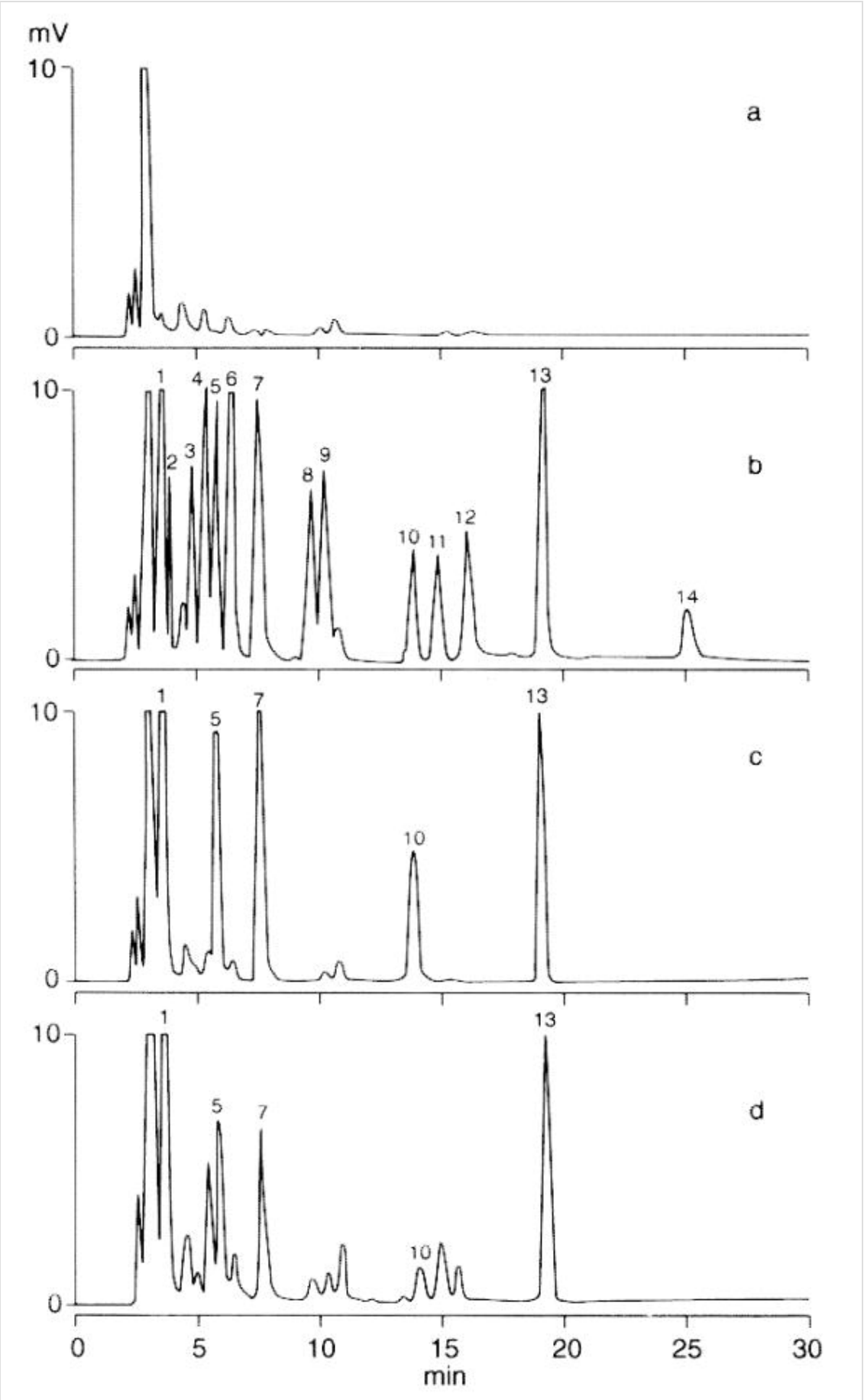
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**FIG. 2. a:** Blank xanthine-free urine. **b:** Xanthine-free urine spiked with caffeine and 13 metabolites in concentrations of 150 µ*M*; 100 µl of 160 µ*M* internal standard was added to the sample before extraction. **c:** Extraction of standard mixture added to xanthine-free urine in concentrations of 150 µ*M*; 100 µl of 160 µ*M* internal standard (peak 13) was added to the sample before extraction. **d:** A sample of 40 µl urine from a healthy volunteer 6 h after ingestion of 200 mg caffeine. Metabolite concentrations: peak 1, 113 µ*M*; peak 5, 97 µ*M*; peak 7, 89 µ*M*; peak 10, 49 µ*M*; 100 µl of 160 µ*M* internal standard (peak 13) was added to the sample before extraction. In all, the 14 consecutively numbered peaks represent AFMU, 7MU, 3MU, 7MX, 1MU, 3MX and 37DMU, 1MX, 13DMU, 37DMX, 17DMU, 17DMX, 13DMX, [beta]-OH-ET (internal standard), and 137TMX, respectively.

#### Recovery

The absolute recoveries of the metabolites were assessed (n = 10) at five concentration levels by comparing the peak area after extraction with the peak area obtained from direct injection of equivalent quantities of pure standard. The five concentration levels were 25, 50, 100, 200, and 400 µ*M*. The recoveries were 62% (57-69%), 79% (67-98%), 94% (84-114%), and 90% (81-108%) for AFMU, 1MU, 1MX, and 17DMU, respectively, given as mean values and ranges for the five concentration levels. The recovery of the internal standard was 86%.

#### Linearity

The linearity of detector response to different concentrations of each compound was determined at concentrations of 25, 50, 100, 200, and 400 µ*M*. The standard curves for the four metabolites went through the origin and were linear over the investigated concentration range. The *r* values of the standard curves were 0.9999, 0.9962, 0.9997, and 0.9998 for AFMU, 1MU, 1MX, and 17DMU, respectively.

#### Repeatability

The intraday repeatability of the method was evaluated by analysis (n = 10) of urine samples spiked to five concentrations: 25, 50, 100, 200, and 400 µ*M*. The coefficients of variation were 2% (1-4%), 3% (1-8%), 2% (1-4%), and 2% (1-3%) for AFMU, 1MU, 1MX, and 17DMU, respectively, given as mean values and ranges for the five concentration levels. The coefficient of variation for the internal standard was 3%.

#### Reproducibility

Interday reproducibility was assessed for 5 days by analysis of urine samples spiked to concentrations of 37.5, 150, and 300 µ*M* for all four metabolites. The coefficients of variation are shown in Table 1.

| | 37.5 μ*M* | 150 μ*M* | 300 μ*M* |
|---|---|---|---|
| AFMU | | | |
| Mean | 36.8 | 147.8 | 294.4 |
| CV (%) | 6 | 2 | 3 |
| Dev. (%) | 2 | 1 | 2 |
| 1MU | | | |
| Mean | 37.2 | 148.8 | 299.8 |
| CV (%) | 5 | 2 | 2 |
| Dev. (%) | 1 | 1 | 0.1 |
| 1MX | | | |
| Mean | 38.6 | 149.7 | 299.2 |
| CV (%) | 7 | 2 | 2 |
| Dev. (%) | 3 | 0.2 | 0.3 |
| 17DMU | | | |
| Mean | 38.6 | 149.2 | 297.8 |
| CV (%) | 6 | 3 | 2 |
| Dev. (%) | 3 | 1 | 1 |
| CYP1A2 ratio[b] | | | |
| Mean | 3.10 | 3.05 | 2.99 |
| CV (%) | 4 | 4 | 3 |
| Dev. (%) | 3 | 1 | 0.3 |

[a] Samples spiked in xanthine-free urine were analyzed once a day for 5 days. The table shows means, coefficients of variation (CV) (%), and deviations from spiked value (Dev.) (%).
[b] Expected CYP1A2 ratio: 3.00.

**TABLE 1.** *Reproducibility and accuracy of 5-acetylamino-6-formylamino-3-methyluracil (AFMU), 1-methyluric acid (1MU), 1-methylxanthine (1MX), and 1,7-dimethyluric acid (17DMU) and of the CYP1A2 ratio*[a]

#### Accuracy

Samples spiked to concentrations of 37.5, 150, and 300 μ*M* were analyzed once a day for 5 days. The mean estimate and deviation from the spiked value are shown in Table 1. The CYP1A2 ratio, AFMU + 1MU + 1MX)/17DMU, was calculated at each concentration level (expected value at each level: CYP1A2 ratio = 3.0). The mean estimate, coefficients of variation, and deviation from the spiked value are shown in Table 1.

#### Limit of Detection and Quantification

The limit of detection, based on a signal-to-noise ratio of 3:1, was 1 μ*M* for AFMU, 1MU, and 1MX and 2 μ*M* for 17DMU. The limit of quantification based on a coefficient of variation <20% for repeated analysis (n = 10) was 2 μ*M* for AFMU and 1MX and 3 μ*M* for 1MU and 17DMU.

#### Biological Validation

In the urine samples from the 277 volunteers, the metabolite concentration ranges were 2.1-327 µ*M*, 4.0-744 µ*M*, 4.9-598 µ*M*, and 6.4-260 µ*M* for AFMU, 1MU, 1MX, and 17DMU, respectively. Standard curves with all four metabolites in the concentrations of 10, 25, 50, 150, and 400 µ*M* were used, and only a very few samples had metabolite concentrations <10 µ*M* or >400 µ*M*.

The log CYP1A2 ratio showed a unimodal distribution (Fig. 3), without any sign of skewness or outliers, and the median value was 4.1 (range 0.91-15.2). The xanthine oxidase ratio was also unimodally distributed (Fig. 3) with one outlier in the upper extreme, and the median value was 0.94 (range 0.08-3.8). The ratio assessing acetylator status showed a bimodal distribution, with 56% slow acetylators (95% confidence limit: 50;62%) and 44% fast acetylators (95% confidence limit: 38;50%) separated by an antimode of 0.49 (Fig. 3). The median was 0.18 (range 0.05-0.44) for slow acetylators and 0.96 (range 0.49-6.4) for fast acetylators.

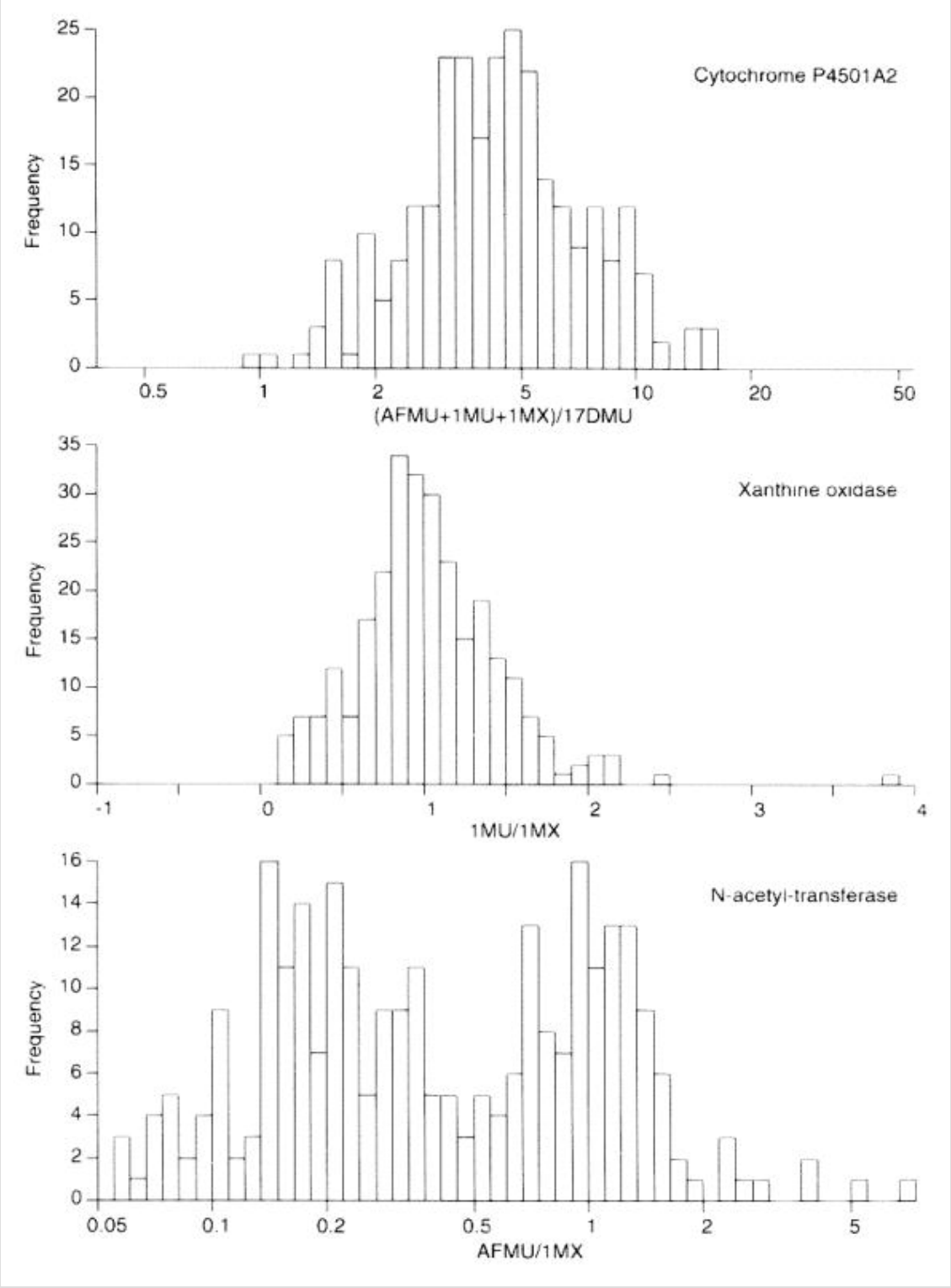


**FIG. 3.** Frequency distribution of the urinary metabolic ratios reflecting CYP1A2, xanthine oxidase, and *N*-acetyltransferase activities in 277 healthy subjects.

The CYP1A2 ratio was significantly lower in women than in men (median difference [95% confidence interval]: -0.6 [-1.1;-0.1]) (Table 2 and Fig. 4) when all smokers and oral contraceptive users were excluded. However, no gender-related difference was seen among smokers (-0.8 [-3.3;0.6]) when oral contraceptive users were excluded. The CYP1A2 ratio was markedly increased in smoking men (2.9 [1.8;4.5]) and in smoking women not using oral contraceptives (2.6 [1.4;4.2]) compared with nonsmoking men and nonsmoking women not using oral contraceptives, respectively, whereas no statistically significant increase in CYP1A2 ratio was seen among smoking women using oral contraceptives (0.4 [-1.1;2.2], compared with nonsmoking women using oral contraceptives (Table 2 and Fig. 4). In women using oral contraceptives, the CYP1A2 ratio was significantly lower than in women not using oral contraceptives (-1.1 [-1.5;-0.6] for nonsmokers and -3.3 [-6.3;-0.9] for smokers) (Table 2 and Fig. 4). For *N*-acetyltransferase and xanthine oxidase ratios, no differences were seen in terms of gender, smoking habits, or the use of oral contraceptives (data not shown).

| | Men | | Women | | | |
|---|---|---|---|---|---|---|
| | | | − oral contraceptives | | + oral contraceptives | |
| | Nonsmokers | Smokers | Nonsmokers | Smokers | Nonsmokers | Smokers |
| Number | 124 | 19 | 52 | 18 | 60 | 4 |
| Median | 4.3 | 8.0 | 3.8 | 6.4 | 2.8 | 3.2 |
| Range | 0.9–13.7 | 4.7–15.2 | 1.5–14.9 | 3.4–11.2 | 1.0–7.9 | 1.8–5.3 |

**TABLE 2.** *Effect of gender, smoking, and oral contraceptive use on CYP1A2 activity, expressed as median values and ranges of the CYP1A2 ratio*

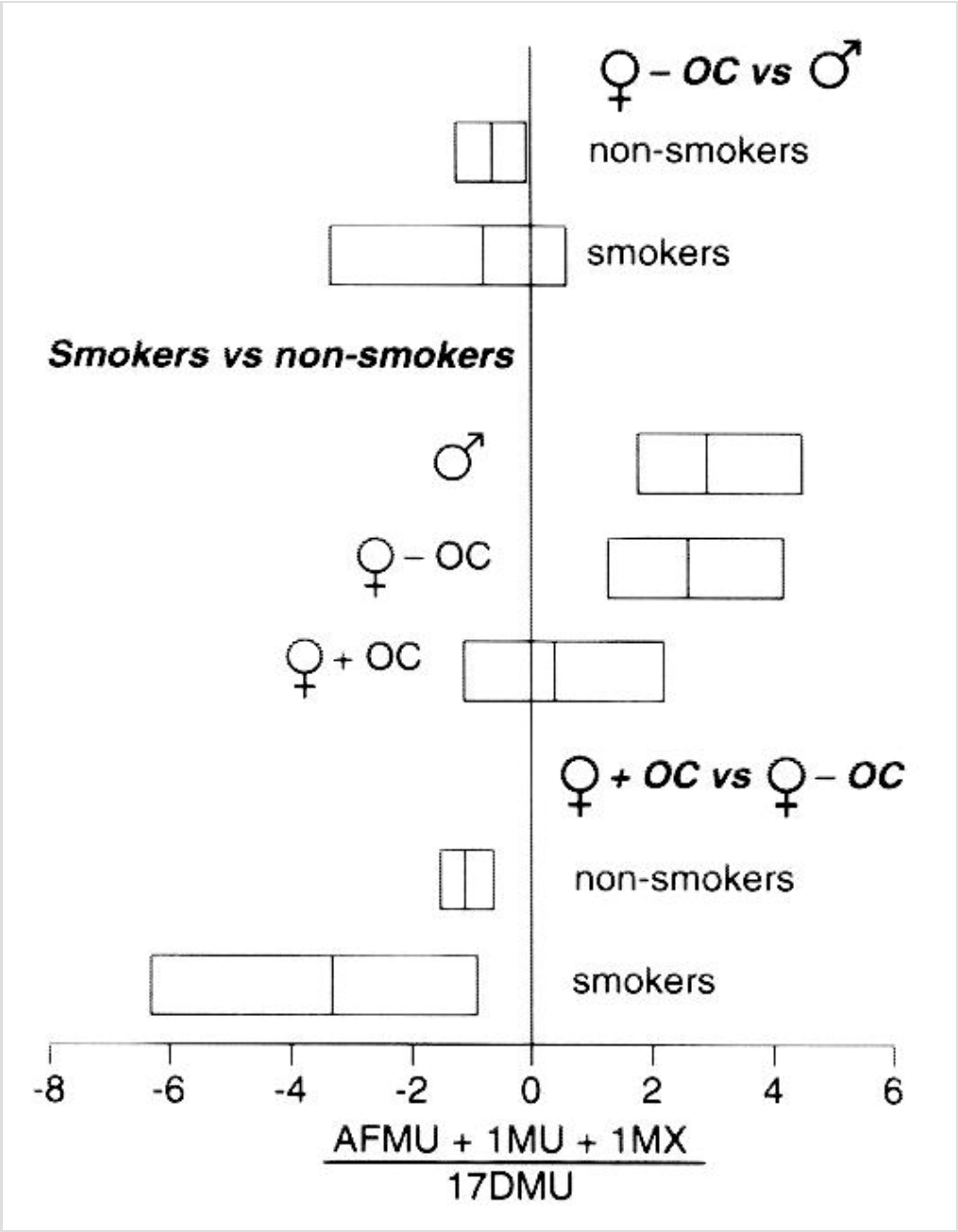


**FIG. 4.** Median differences and 95% confidence intervals in the urinary metabolic ratios expressing CYP1A2 activity in 277 healthy subjects stratified by gender, smoking habits, and use of oral contraceptives (OC).

Among the 277 participants, eight subjects (2.8%) were classified as poor metabolizers of *S*-mephenytion (mephenytoin *S/R* ratio of 0.97:1.4), whereas 268 were classified as extensive metabolizers (mephenytoin *S/R* ratio of 0.1:0.59). For one subject an *S/R* ratio could not be determined owing to low *S*- and *R*-mephenytoin levels in the urine. A weak correlation was found between the CYP1A2 ratio and the mephenytoin *S/R* ratio ($r_s$ = 0.13; p = 0.035), whereas no correlation was found between *N*-acetyltransferase and xanthine oxidase and the mephenytoin *S/R* ratio ($r_s$ = -0.07 and -0.03, respectively; p > 0.05).

Twenty-one subjects (7.6%) were classified as poor metabolizers of sparteine (sparteine MR of 20:1,800), and 256 could be assigned to the extensive metabolizer phenotype group (MR of 0.04:19). There was no correlation between the CYP1A2, *N*-acetyltransferase, and xanthine oxidase ratios and the sparteine MR ($r_s$ = -0.001-0.03; p > 0.05).

## DISCUSSION

This study describes an isocratic, reverse-phase HPLC method developed for simultaneous determination of four caffeine metabolites in human urine. By the substitution of the highly toxic organic solvent chloroform with the less hazardous solvent ethyl acetate, occupational safety in the laboratory has been improved compared with earlier published HPLC methods (18-20). Our method provides an analytically valid tool for assessing CYP1A2, *N*-acetyltransferase, and xanthine oxidase activities in humans, since it evidences good overall recovery, small variations, and low detection limits for all metabolites.

The caffeine dose of 200 mg, corresponding to two cups of strong coffee, was well accepted by most volunteers. A few subjects, who did not commonly drink coffee or tea, complained of insomnia. To minimize this side effect, it is recommended that caffeine tests be performed early in the day. No other side effects were reported.

The CYP1A2 ratio was markedly increased in smoking subjects, confirming the expected effect of induction of CYP1A2 by tobacco smoking (18,22,31-34). By analogy, the CYP1A2 ratio was, as expected, statistically significantly lower in women using oral contraceptives than in women not using oral contraceptives (18,35,36); this finding is probably due to inhibition of CYP1A2 by oral contraceptives. The CYP1A2 ratio was slightly, but statistically significantly lower in women than in men, even when smokers and oral contraceptive users were excluded. This finding is in agreement with the findings in a similar study (31), whereas the gender-related difference was not found in other, smaller studies (18,19,22,26).

Our results are in agreement with the finding that the clearance of the CYP1A2 substrate, clozapine (6-8), is lower in women than in men (37,38). However, the effect of gender on CYP1A2 activity seems to be weak, suggesting that large sample sizes are necessary in order to avoid a statistical type 2 error. The CYP1A2 ratio shows a remarkably large variability, even within subgroups of individuals (Table 2), suggesting that CYP1A2 activity is influenced by factors other than gender, smoking status, and use of oral contraceptives, as evaluated in this study. It is possible that CYP1A2 activity is also controlled by genetic factors. This hypothesis is the subject of ongoing studies in our laboratory.

A statistically significant, albeit very weak correlation was found between the CYP1A2 ratio and the mephenytoin *S/R* ratio. There have not been any previous reports on a link between CYP1A2 activity and the ability to hydroxylate *S*-mephenytoin, reflecting CYP2C19. The subject requires further investigation, but with our present knowledge it is a puzzle that cannot be explained as anything but a type 1 error. Our study confirms all of the known characteristics of CYP1A2 activity, and even though the CYP1A2 ratio has its shortcomings, owing to the complex metabolism of caffeine and the involvement of other P450s (39,40), the ratio seems to be a reliable measure of CYP1A2 activity. Furthermore, the results obtained for *N*-acetyltransferase and xanthine oxidase were all in agreement with earlier reports (22,26,31,41).

It is concluded that our HPLC method is analytically and biologically reliable and thus is a useful tool for assessing CYP1A2, *N*-acetyltransferase, and xanthine oxidase activities both in individuals and in populations. It is also valuable for determining CYP1A2 activity in patients treated with CYP1A2 substrates, for whom clinical dose titration is not feasible. In addition, the test will be valuable for the identification of new CYP1A2 substrates.

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Key Words: High-performance liquid chromatography; Cytochrome P4501A2; Caffeine; Metabolic ratios

## IMAGE GALLERY

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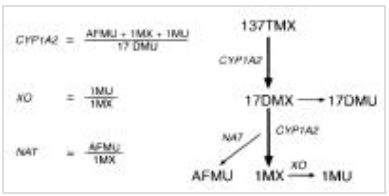


Fig. 1

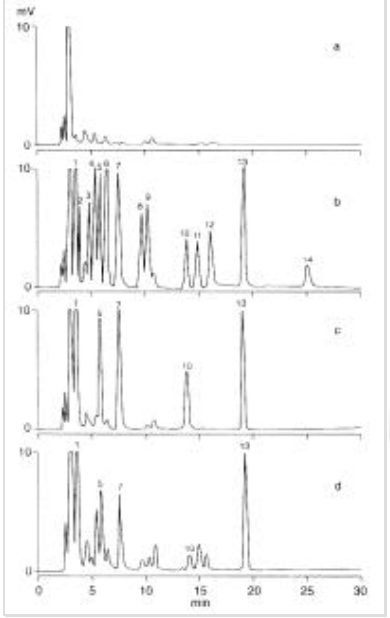


Fig. 2

| | 37.5 μM | 150 μM | 300 μM |
|---|---|---|---|
| AFMU | | | |
| Mean | 36.8 | 147.8 | 294.4 |
| CV (%) | 6 | 2 | 3 |
| Dev. (%) | 2 | 1 | 2 |
| 1MU | | | |
| Mean | 37.2 | 148.8 | 299.8 |
| CV (%) | 5 | 2 | 2 |
| Dev. (%) | 1 | 1 | 0.1 |
| 1MX | | | |
| Mean | 38.6 | 149.7 | 299.2 |
| CV (%) | 7 | 2 | 2 |
| Dev. (%) | 3 | 0.2 | 0.3 |
| 17DMU | | | |
| Mean | 38.6 | 149.2 | 297.8 |
| CV (%) | 6 | 3 | 2 |
| Dev. (%) | 3 | 1 | 1 |
| CYP1A2 ratio[b] | | | |
| Mean | 3.10 | 3.05 | 2.99 |
| CV (%) | 4 | 4 | 3 |
| Dev. (%) | 3 | 1 | 0.3 |

[a] Samples spiked in xanthine-free urine were analyzed once a day for 5 days. The table shows means, coefficients of variation (CV) (%), and deviations from spiked value (Dev.) (%).
[b] Expected CYP1A2 ratio: 3.00.

Table 1

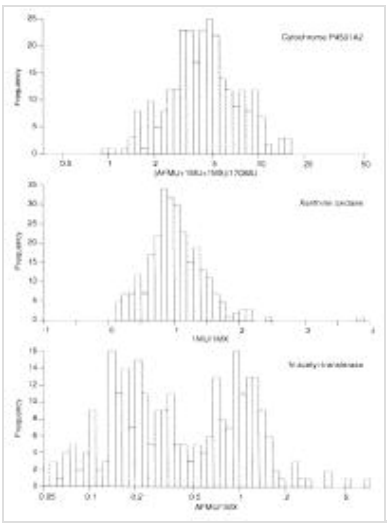

Fig. 3

| | Men | | Women | | | |
|---|---|---|---|---|---|---|
| | | | – oral contraceptives | | + oral contraceptives | |
| | Nonsmokers | Smokers | Nonsmokers | Smokers | Nonsmokers | Smokers |
| Number | 124 | 70 | 52 | 18 | 40 | 4 |
| Median | 6.1 | 8.8 | 3.8 | 6.4 | 2.8 | 3.2 |
| Range | 0.9–12.1 | 4.7–17.2 | 1.5–14.9 | 3.4–11.2 | 1.0–7.9 | 1.9–5.3 |

Table 2

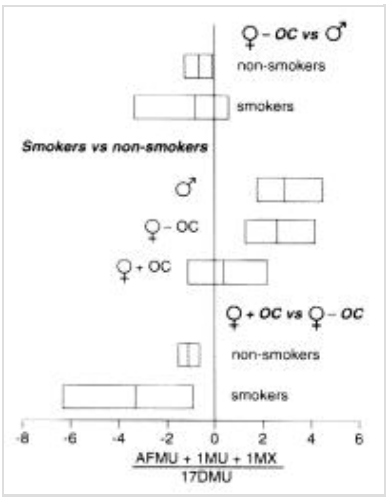


Fig. 4

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