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Determination of N-acetylator phenotype using caffeine as a probe compound: a comparison of high-performance liquid chromatography and capillary electrophoresis methods

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ABSTRACT

A test for determining N-acetylator metabolic phenotype has been developed using caffeine as a probe drug. A spot sample of urine is taken, and the unextracted urine is then analysed by micellar electrokinetic capillary chromatography. Phenotype is determined from the peak-area ratio of urinary 5-acetylamino-6-formylamino-3-methyluracil to 1-methylxanthine. Phenotype assignments using this method were compared with those made using a standard high-performance liquid chromatography assay, with good agreement between the two methods. The advantage of the capillary electrophoresis analysis is that no sample extraction is necessary, resulting in a total analysis time of around 20 min, and removing a potential source of error.

INTRODUCTION

A genetic polymorphism in the N-acetylation metabolic pathway affects the metabolism of a number of drugs [1]. An individual's phenotype may be determined by administering a drug which is metabolised by this pathway, and measuring the plasma or urinary ratio of the drug and/or metabolites. Several probe drugs have been used for the determination of the N-acetylator metabolic phenotype; a simple method for phenotyping was proposed by Grant *et al.* [2], involving the determination of the urinary ratio of two caffeine metabolites, 5-acetylamino-6-formylamino-3-methyluracil (AFMU) and 1-methylxanthine (1X), measured between 2 and 6 h after caffeine ingestion. Comparison with an acetylator phenotyping test using sulfamethazine as a probe drug and a phenotyping test using caffeine and monitoring several caffeine metabolites showed a good correlation between the assigned phenotypes for each method [3]. More recently, it has been shown that a simple measurement of the high-performance liquid chromatography (HPLC) peak-height ratio of AFMU to 1X is adequate for a phenotyping test [4], and this cuts out the step of making a calibration curve to quantitate each analyte. It has been noted by Tang *et al.* [3] that AFMU may undergo spontaneous deformylation in urine samples, giving 5-acetylamino-6-amino-3-methyluracil (AAMU). These authors suggest that it is perhaps better to first convert AFMU to AAMU by basification of the sample to assist degradation, and then determine the AAMU/1X ratio. We have taken the approach of immediately acidifying urine sam-

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ples upon collection, to arrest any degradation of AFMU.

In the work by Grant *et al.* [2], metabolite concentrations were determined by HPLC, after liquid–liquid extraction of AFMU and 1X from urine. This was reported to be a reliable and accurate method for the determination of N-acetylation phenotype, but the extraction step makes the process rather time-consuming. The HPLC peak-height method [4] also requires an extraction before analysis. We are interested in performing large-scale prospective phenotyping of subjects entered on a number of clinical trials, and therefore were interested in developing a quicker, less labour-intensive method of phenotyping. To try and avoid having to perform an extraction step, we decided to investigate the use of capillary electrophoresis (CE) [5] instead of HPLC as an analytical method for AFMU and 1X in urine. A review of some biomedical and clinical applications of CE has recently been published [6]. We have been using CE for a number of drug assays [7,8], and in particular the micellar electrokinetic capillary chromatographic (MECC) variant of this technique [9] has been shown to be useful for analyses with direct injection of biological samples [8,10]. There are a number of previous reports of the analysis of caffeine and related compounds by CE. Both capillary zone electrophoresis (CZE) [11] and MECC [11,12] analyses have been demonstrated. Thormann *et al.* [12] have shown that clinically useful determinations of caffeine and theophylline in body fluids can be made using MECC with UV absorbance detection. They reported the quantitation of caffeine and theophylline with direct injection of plasma and saliva, although they found that an extraction step was necessary for the analysis of these compounds in urine. Atamna *et al.* [11] suggested that the sensitivity of CE methods with UV absorbance detection would be inadequate for the determination of caffeine metabolites in biological fluids. This conclusion seems to have been unduly pessimistic, at least for the analysis of AFMU and 1X.

Here, we report a phenotyping method using MECC to determine AFMU/1X ratios in urine, and compare the phenotype assignments from this method with those from on HPLC analysis. As in the HPLC peak-height method [4], no attempt is made to determine the molar ratio of AFMU to 1X. In this case the peak-area ratio AFMU/1X determined by UV absorption at 280 nm is used to assign phenotypes. There is good agreement between the phenotype assignments determined by HPLC and CE. The principal advantage of the CE method is that no sample extraction is required. This means that potential errors introduced by the extraction are avoided, and the whole procedure for phenotype determination by CE takes less than 20 min.

EXPERIMENTAL

Subjects

Urine samples were provided by healthy volunteers. Those who normally drink caffeinated beverages were not limited in their caffeine intake. Subjects with a normally caffeine-free diet were given a cup of coffee or a cola beverage. All subjects were asked to provide a single-spot urine sample any time between 2 to 4 h after consuming a caffeinated drink. The pH of urine samples was adjusted to ~ 3.5 with glacial acetic acid after collection to stop decomposition of AFMU to AAMU. Urine samples were then stored at -20°C until analysis.

Sample extraction

The method used was modified from that described by Grant *et al.* [2] in that no internal standard was added. Ammonium sulfate was added to acidified urine at a concentration of 600 mg ml^{-1}, and the urine was vortex-mixed for 5 s. A 0.2-ml volume of the urine was then placed in a 15-ml centrifuge tube. A 6-ml volume of chloroform–isopropanol (95:5) was added to the tube and the tube was vortex-mixed for 1 min. After centrifugation (1000 *g* for 5 min), the organic phase was transferred to another tube and evaporated to dryness under a stream of air at room temperature. The residue was resuspended with 0.2–0.5 ml of 0.05% acetic acid.

Capillary electrophoresis

Separations were carried out using either Applied Biosystems (Toronto, Canada) Model 270A or Model 270A-HT CE instruments. The separation capillaries were 72 cm long (50 cm to the detector), with an internal diameter of 50 μm (Polymicro Technologies, Phoenix, AZ, USA). The separation buffer for analysis of urine samples and extracts was made by mixing solutions of 100 m*M* sodium borate and 100 m*M* sodium dihydrogenphosphate to give a pH of 8.43, as measured using a Fisher Accumet 915 pH meter with a Model 13-620 Ag/AgCl electrode (Fisher, Pittsburgh, PA, USA). This buffer was then mixed with a 200 m*M* solution of sodium dodecyl sulfate (SDS) (Sigma, St. Louis, MO, USA) in a buffer/SDS ratio of 13:7, giving a final SDS concentration of 70 m*M*. For investigation of the effect of buffer type and pH, phosphate buffers were made by mixing 25 m*M* sodium dihydrogenphosphate and disodium hydrogenphosphate solutions to give the required pH, whilst 20 m*M* sodium borate and 20 m*M* Tris solutions were adjusted to the required pH with 1 *M* hydrochloric acid. Hydrodynamic injection was performed by applying a vacuum of 17 kPa at the cathode end of the capillary. For analysis of urine samples, vacuum was normally applied for 1 s, although sometimes longer injections (up to 5 s) were used if there were low levels of the metabolites present. For analysis of extracted urine, 5-s vacuum injections were used. Detection was performed by on-capillary UV absorbance measurements, usually at a wavelength of 280 nm, with a detection time-constant of 0.1 s. Before injection onto the CE instrument, all samples were filtered through disposable 0.45-μm-pore membranes. The capillary was rinsed with 0.1 *M* sodium hydroxide for 1 min and with buffer solution for 3 min before each analysis. A separation potential of 17 kV was used with the phosphate–borate–SDS buffer system, leading to a current flow of 40 μA.

High-performance liquid chromatography

The extracts were analyzed by a modified version of the method described by Grant *et al.* [2]. The HPLC system consisted of a solvent delivery pump (Applied Biosystems Model 400, Toronto, Canada), an Ultremex C_{18}, 3 μm particle size, 150 mm × 4.6 mm I.D. column (Phenomenex, Torrance, CA, USA), a variable-wavelength detector (Applied Biosystems Model 783) set to 280 nm and an integrator (Shimadzu Model CR3A, Columbia, MD, USA). For multi-wavelength detection, an Applied Biosystems Model 1000S diode-array detector was used. The mobile phase was made up of 0.05% acetic acid–acetonitrile (95:5) at a flow-rate of 0.75 ml min^{-1}.

Chemicals

Standard samples of 1X were obtained from Sigma (St. Louis, MO, USA). Pure AFMU was purchased from Dr. Tang (University of Toronto, Toronto, Canada). All buffers and reagents were obtained from BDH (Toronto, Canada).

RESULTS AND DISCUSSION

The effect of buffer pH and composition on the separation of AFMU and 1X was investigated, as well as the effect of the addition of SDS. Phosphate, borate and Tris buffers were used over a pH range of approximately 7–9. Fig. 1 shows the effective mobility [13] of standard samples of AFMU and 1X as a function of pH for the different buffers with the analyte effective mobility, μ_{eff}, being calculated as the difference between

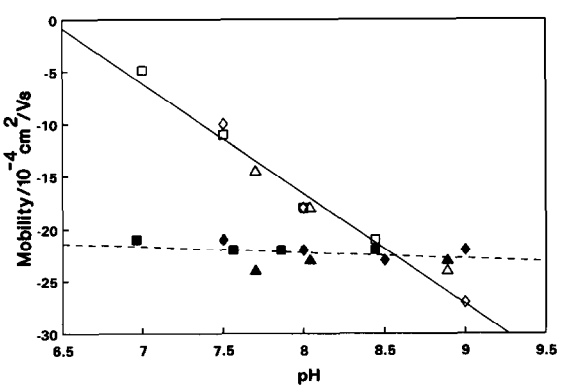

Fig. 1. Variation in effective mobility of AFMU (filled markers) and 1X (open markers) with phosphate (□), borate (△) and Tris (◇) buffers. Separation conditions are given in the text.

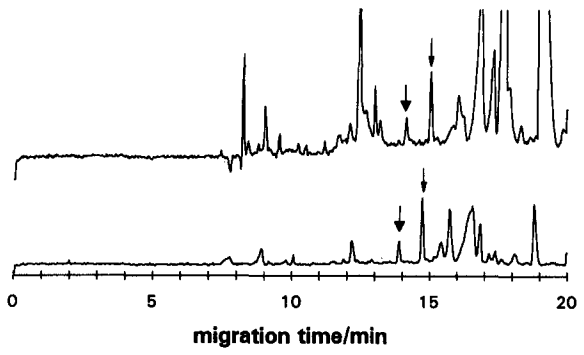

Fig. 2. Superimposed electropherograms of unextracted urine (upper trace) and a urine extract from the same sample (lower trace). The peaks marked with arrows are AFMU (wide arrow) and 1X (narrow arrow). The low AFMU-to-1X ratio shows this subject to be a slow acetylator. Conditions: capillary, 72 cm long (50 cm to detector) fused silica, internal diameter 50 μm; buffer, phosphate–borate, pH 8.43 with 70 m*M* SDS; separation potential, 17 kV; current, 39 μA; detection, UV absorbance at 280 nm; risetime, 0.1 s.

the overall analyte mobility and the electroosmotic flow mobility, μ_{eo}. The marker of electroosmotic flow was taken to be the bottom of the first negative dip in the electropherogram (Fig. 2). It can be seen that whilst the effective mobility of AFMU remains more or less constant over the pH range investigated, the mobility of 1X varies considerably. The effect of addition of SDS to a pH 8.43 phosphate–borate buffer was investigated, up to an SDS concentration of 140 m*M* (Table I). With 17.5 m*M* SDS, there was a slight reduction (3–5%) in electroosmotic flow mobility and in the effective mobility of AFMU and 1X. Further addition of SDS up to 140 m*M* resulted in a small increase in effective mobility, indicating that these compounds are not strongly included in the micelles, and that their motion is primarily due to their negative charge at this pH. This may be explained because the negative charge on AFMU and 1X under the chosen separation conditions will cause repulsion of these compounds from the anionic SDS micelle.

Both untreated urine and urine extracts prepared for HPLC were analysed by CE. Typical electropherograms of untreated urine (upper trace) and of an extract of the same sample prepared for HPLC analysis (lower trace) are shown in Fig. 2. Two peaks are identified by arrows. The first of these is AFMU, the second is 1X. The off-scale peak at around 19 min in the electropherogram of untreated urine is due to uric acid, and because of its prominence it was a useful marker to follow during method development. It can be seen that there is a good resemblance between the two electropherograms. As would be expected, fewer peaks are seen in the extracted sample, but in the region where AFMU and 1X appear there is little difference in the electropherograms, and it is concluded that the extraction aids little in removing any potential interferences in the CE analysis. The untreated urine was injected by a 1-s application of vacuum, whilst the extract was injected with a 5-s vacuum. As can be seen from the behaviour of standard samples shown in Fig. 1, the migration of 1X could be greatly affected

TABLE I

EFFECT OF ADDITION OF SDS ON THE ELECTROOSMOTIC FLOW MOBILITY, AND EFFECTIVE MOBILITIES OF AFMU AND 1X

Buffer, phosphate–borate, pH 8.43.

SDS concentration (m*M*)	$\mu_{effAFMU}$ (10^{-6} cm^2 V^{-1} s^{-1})	μ_{eff1X} (10^{-6} cm^2 V^{-1} s^{-1})	μ_{eo} (10^{-6} cm^2 V^{-1} s^{-1})
0	−209.0	−215.9	512.2
17.5	−197.6	−209.0	494.3
35	−200.2	−211.0	496.6
70	−203.0	−218.1	489.8
140	−223.8	−243.3	486.0

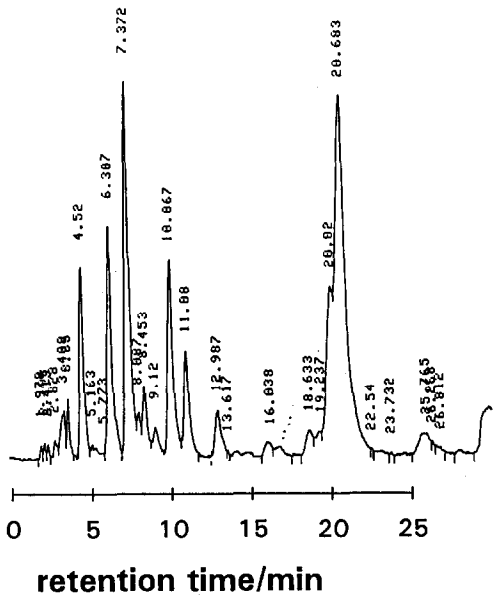

Fig. 3. HPLC of a urine extract, from the same sample as shown in Fig. 2. AFMU elutes at 4.5 min and 1X elutes at 10.1 min. Separation conditions: column, C_{18}, 150 mm × 4.6 mm I.D.; mobile phase, 0.05% acetic acid–acetonitrile (95:5); flow-rate, 0.75 ml min^{-1}; detection UV absorbance at 280 nm.

by even small changes in pH. At pH 9.3, 1X eluted at around 20 min, and was partially overlapped with other urine components. As the pH was reduced, the 1X peak was seen to migrate more quickly. The final separation conditions were chosen because 1X migrated in an relatively interference-free portion of the electropherogram. The presence of SDS in the buffer system for the analysis of urine is important, since it affects the migration of urine components other than AFMU and 1X. Without surfactant there are interfering peaks which are partially overlapped with 1X. Under the chosen separation conditions, the effective mobilities of AFMU and 1X were found to be $-2.08 \cdot 10^{-4}$ and $-2.22 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1}, respectively (migration time and mobility reproducibility are discussed further below). In standard samples of AFMU, a peak was sometimes observed at around 11.5 min (effective mobility $-1.5 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1}), which was attributed to AAMU, the degradation product of AFMU. A HPLC chromatogram for a urine extract is shown in Fig. 3. This is a chromatogram of the same extract as shown analysed by CE in Fig. 2. In the reversed-phase HPLC system AFMU elutes relatively quickly, after around 4.5 min, whilst 1X is more retained, eluting after 10 min.

The high efficiency of the CE separation allows the rapid resolution of a large number of components in unextracted urine samples. However, the complexity of the urine electropherograms does make peak identification and assignment a complex process. To locate AFMU and 1X, urine samples were spiked with pure standards under a variety of separation conditions. Blank urines were also obtained from a number of volunteers who refrained from caffeine ingestion for at least 24 h and these were compared with their samples after caffeine ingestion. To further confirm the identity the 1X and AFMU peaks, spectra were taken on-line by repeatedly and automatically reversing the applied electric field, thus migrating the sample back and forth through the detector, whilst changing the detection wavelength [14]. Thus absorbance measurements on the sample could be taken at a number of wavelengths during one analytical run, and these could be com-

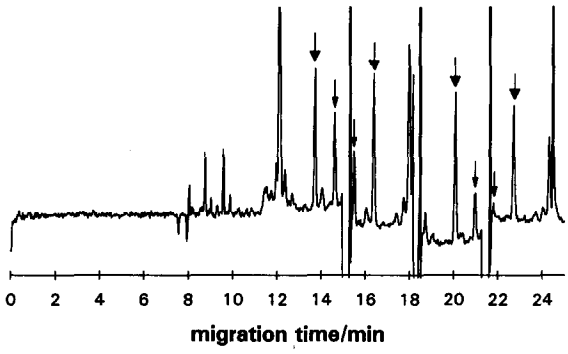

Fig. 4. Time- and wavelength-programmed electropherogram of urine. AFMU peaks are marked by wide arrows, 1X by narrow arrows. Up until 15 min the separation proceeds with a potential of +17 kV at the inlet end of the capillary, with detection at a wavelength of 280 nm. The detection wavelength is then changed to 285 nm, and the electric field reversed, which causes the analytes to migrate back through the detector. This process is repeated twice more, with detection at 290 and 300 nm. The off-scale positive and negative peaks during the electropherogram are due to the detector re-zeroing after changes in wavelength. Separation conditions as in Fig. 2.

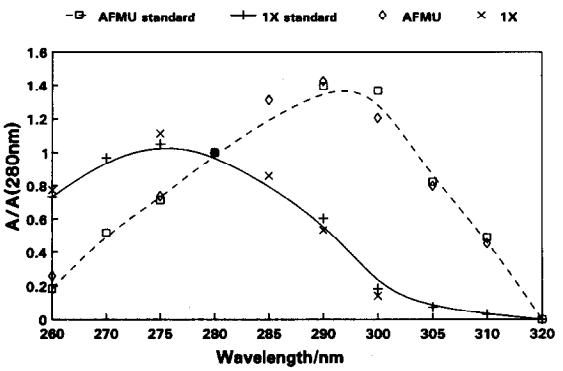

Fig. 5. Absorbance spectra for AFMU and 1X, with absorbances normalised to 280-nm values. Curves represent a fitting to the AFMU (□) and 1X (+) absorbances obtained from electrophoresis of aqueous standard samples of these compounds. Data points for AFMU in urine (◇) and 1X in urine (×) are in excellent agreement with the standard spectra.

pared with results obtained in the same fashion from standard samples of the target compounds. Fig. 4 shows a typical voltage- and wavelength-programmed electropherogram of a urine sample, and Fig. 5 shows the spectra obtained in this fashion. The spectra are normalised to the absorption of each compound at 280 nm, a wavelength at which both compounds give good responses. It can be seen that there is a pleasing agreement between the spectra from the urine and standard samples, further confirming the identity and purity of the 1X and AFMU peaks. Whilst the use of real-time multi-wavelength detection is obviously a simpler solution if available [12], voltage- and wavelength-programming allowed us to achieve a similar result in identifying the peaks of interest. On-line spectra were also obtained during some of the HPLC analyses, using a diode-array UV absorbance detector. These revealed that there is a shift of over 10 nm in the wavelength of the absorbance maximum of AFMU measured in the HPLC mobile phase (λ_{max} = 283 nm) and the CE buffer ($\lambda_{max} \approx$ 295 nm). Therefore, HPLC and CE peak-area ratios of AFMU/1X are not equal, and thus cannot be directly compared for phenotype assignment. Occasionally, peak identification was a problem in the CE analysis, due to a large number of closely migrating peaks. In such cases, confirmation was made either by spiking with standard samples, or

TABLE II

INTRA-DAY REPRODUCIBILITY DATA FOR MIGRATION TIME AND EFFECTIVE MOBILITY, AND AFMU/1X FOR ONE FAST AND ONE SLOW ACETYLATOR SUBJECT; INTER-DAY REPRODUCIBILITY FOR MIGRATION TIME AND EFFECTIVE MOBILITY FOR TEN RANDOMLY CHOSEN SUBJECTS WITH DATA TAKEN OVER A PERIOD OF SIX WEEKS

Values in parentheses are coefficients of variation.

	Slow acetylator	Fast acetylator	Inter-day
n	7	10	10
t_{eo} (min)	7.46 ± 0.01 (0.14%)	7.62 ± 0.01 (0.14%)	7.63 ± 0.08 (1.0%)
μ_{eo} (10^{-6} cm^2 V^{-1} s^{-1})	473.6 ± 0.7 (0.14%)	463.5 ± 0.7 (0.14%)	462.7 ± 4.9 (1.1%)
t_{AFMU} (min)	13.37 ± 0.01 (0.11%)	13.81 ± 0.04 (0.30%)	13.88 ± 0.24 (1.7%)
$\mu_{effAFMU}$ (10^{-6} cm^2 V^{-1} s^{-1})	−209.5 ± 0.5 (0.23%)	−207.8 ± 0.3 (0.14%)	−208.2 ± 2.6 (1.2%)
t_{1X} (min)	13.77 ± 0.08 (0.61%)	14.60 ± 0.04 (0.27%)	14.69 ± 0.30 (1.9%)
μ_{eff1X} (10^{-6} cm^2 V^{-1} s^{-1})	−217.1 ± 1.3 (0.60%)	−219.9 ± 1.2 (0.55%)	−222.2 ± 3.5 (1.6%)
C.V. of ratio AFMU/1X	7.7%	4.8%	

reanalysing using a detection wavelength of 300 nm and observing the change in response to the AFMU and 1X peaks. So far no drug interference tests have been made, since we have been using this method for prospective phenotyping in clinical trials before the administration of any medication.

Intra-day reproducibility of the CE method was determined for one fast and one slow acetylator subject. The variability in the AFMU/1X peak-area ratio was determined, as well as the variability in migration time. Because variations in the electroosmotic flow can give rise to considerable variation in the migration time of analytes in CE, the variability in the effective analyte mobility was also calculated for AFMU and 1X [13]. Data for reproducibility of migration time, electroosmotic flow mobility and analyte effective mobility is shown in Table II. Mean values and standard deviations are given, as well as coefficients of variation. Intra-day reproducibility of data for one slow and one fast acetylator subject is shown, and inter-day reproducibility in migration time and mobility is shown for analyses of ten randomly chosen samples from various subjects which were made over a period of six weeks. The inter-day data were taken during a period when over 200 analyses of extracted and unextracted urine samples were made using the same capillary. During this time, no overall trend in the variation in electroosmotic flow or analyte mobility was seen. This suggests that there is little or no modification of the capillary surface by injection of untreated urine samples when using this buffer system. It can be seen from the reproducibility data in Table II that whilst the intra-day results indicate that there is no obvious advantage in determining the effective mobility over measurement of migration time, the inter-day data do show somewhat less variability in the mobility values than in the migration times. Therefore, calculation of the migration time knowing the effective mobility and electroosmotic flow mobility is probably a better method of peak identification than simply making assignments on the basis of the expected migration time.

If more than ten or so analyses were made at one time, a slow drift in the mobility of 1X relative to that of AFMU or the elctroosmotic flow mobility was sometimes observed. To avoid this drift, it was found to be necessary to change the buffer at the capillary inlet after several analyses when using the 270A CE instrument. The drift was less pronounced using the 270A-HT, which has a larger inlet buffer vial which is capped to avoid evaporation. Presumably the drift is due to ion depletion from the inlet buffer vial. In practice this is not a great problem, since with most commercial CE instruments it is possible to automatically change the inlet buffer after a given number of runs to maintain the required analytical conditions. Perhaps the consistently higher variability in mobility for 1X than AFMU seen in Table II reflects the greater sensitivity of the migration of this compound on the buffer composition under the experimental conditions chosen for the urine analysis.

The CE peak-area ratios for extracted and unextracted urine samples were plotted against the HPLC peak-height ratios for the corresponding subjects. HPLC peak-height measurements were used since closely eluting peaks sometimes lead to difficulties in accurate quantitation of the AFMU and 1X peak areas. The best linear relationship was obtained for the CE ratio of extracted urine plotted against the HPLC peak-height ratio. The line had a slope of 3.01 and an intercept of -0.02, with a correlation coefficient of 0.961 (twenty subjects). This is illustrated in Fig. 6a. The data for the fastest acetylator was excluded from the line-fitting, because for this single subject problems were encountered with the linearity of response of the HPLC system. The plot of the direct-injection CE against HPLC peak-height results had a poorer correlation coefficient (0.929), with a slope of 2.65 and an intercept of -0.13 (line-fitting for the same twenty subjects as above). These data are shown in Fig. 6b. The lower slope with the direct-injection CE results suggested that there was incomplete extraction of AFMU, and that the poorer correlation coefficient may reflect variability associated with the extraction procedure, which could introduce an extra error into the HPLC analysis. Because of

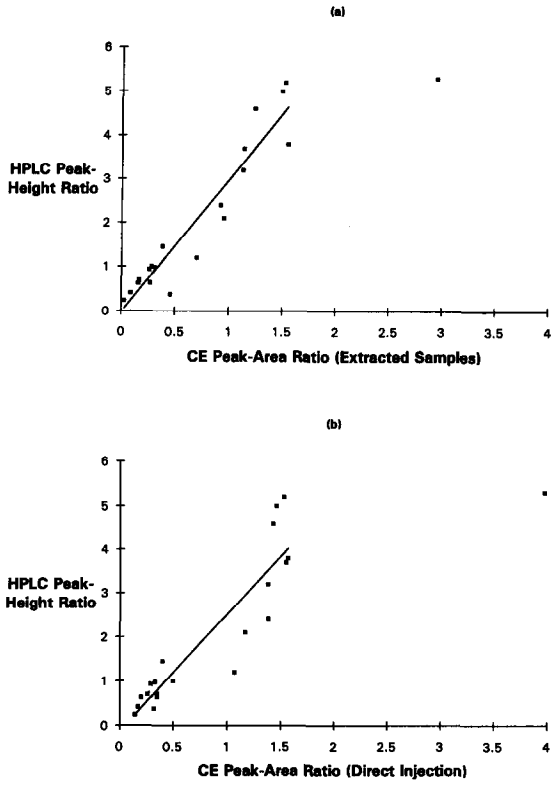

Fig. 6. Comparison of the HPLC peak-height ratios AFMU/1X to CE peak-area ratios. (a) Comparison of extracted samples analysed by each technique; (b) comparison of HPLC of extracted samples with CE measurements of unextracted urine. Lines are fitted to the first twenty data points only, and exclude the data for the subject with the highest AFMU/1X ratio, where problems of non-linearity in the HPLC system were encountered.

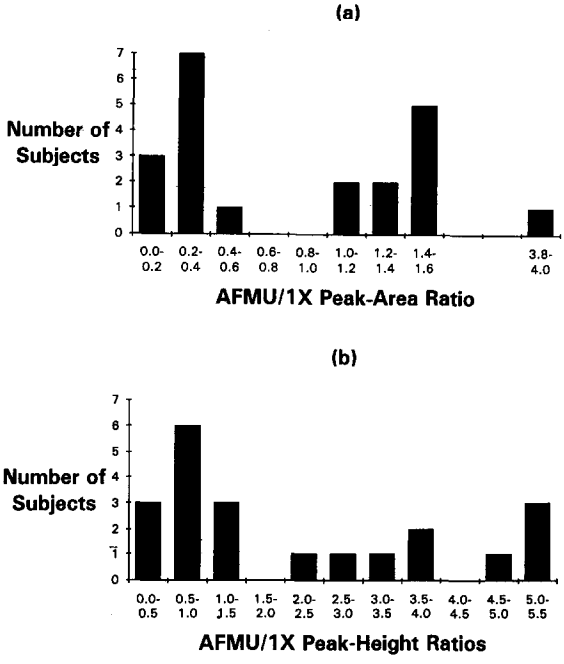

Fig. 7. Histogram showing the distribution of AFMU/1X ratios for twenty-one subjects as determined by (a) CE and (b) HPLC.

this, the performance of the extraction method was examined. Ten extractions of a blank urine sample which had been spiked with AFMU and 1X were made. The extracted samples were each analysed in duplicate by CE, and the unextracted urine was analysed by CE five times. For the extracted samples the analytical precision was good, with coefficients of variation in the peak areas of 5.6% for AFMU and 3.7% for 1X (n = 18). The corresponding values for unextracted urine were 5.2 and 2.7%, respectively (n = 5). However, recovery was found to be poor; 61% for AFMU and 71% for 1X. Because it was not clear that the use of an internal standard would increase the analytical accuracy in determining the peak-area ratio of the extracted AFMU and 1X, we chose to omit the internal standard from the extraction procedure described by Grant *et al.* [2]. El-Yazigi *et al.* [4], who described an HPLC peak-height ratio method for acetylator phenotyping, did not use an internal standard.

Phenotype determinations were made on the basis of ratios of AFMU/1X determined by the CE peak-area ratio, and HPLC peak-height ratios for these two peaks. The distribution of peak-area ratios of AFMU to 1X determined by CE for twenty-one subjects is shown in the histogram (Fig. 7a). Eleven slow acetylators are clearly defined. Ten fast-acetylators were found. Nine of the subjects fitted into an intermediate population (presumably heterozygous fast acetylators), whilst one subject had a considerably higher AFMU/1X ratio and may represent one homozygous fast acetylator within the group. Fig. 7b shows the distribution of peak-height ratios of AFMU/1X determined by HPLC. In this case, twelve subjects are classified as slow acetyla-

tors. For the CE data, an antimode separating slow and fast acetylators can be defined between 0.6 and 1.0. For HPLC peak height measurements, an antimode falls around 1.5–2.0. The measured peak-area or peak-height ratios are to a certain extent dependent on the exact analytical conditions, and could vary when using different instrumentation. Therefore, application of these methods would usually require the intitial determination of the values of the antimodes. One individual classified as slow by HPLC (the fastest thus classified), falls squarely in the group of fast acetylators as determined by CE. The CE values of AFMU/1X for this subject are 1.07 for the direct injection of urine and 0.70 for the extracted sample. Comparing the electropherograms of untreated and extracted urine from this subject, no evidence was found for breakdown of AFMU to AAMU. Therefore, it seems most likely that for this subject poor extraction of AFMU lead to a mis-assignment by the HPLC method. Unfortunately the subject was not available for re-testing. For the fast acetylators, there is a reasonably tight grouping found by CE, with one subject having a very much higher AFMU/1X ratio, possibly representing a homozygous fast acetylator. The HPLC peak-height results showed a wider distribution amongst the fast acetylators, and the one extremely fast acetylator appears grouped with three other subjects. Upon re-examination of the data it was found that in fact the fastest acetylator gave an off-scale response for AFMU on the HPLC system, and thus the determined value represents simply a lower limit for the AFMU/1X ratio.

CONCLUSIONS

A method for the determination of acetylator phenotype using CE has been developed, which has been shown to give assignments which are in agreement with those determined from HPLC peak-height ratios. The advantage of this method over those previously published is that it does not require extraction of urine before analysis. Using CE it has been possible to compare the AFMU/1X ratio in untreated and extracted urine, and it has been shown that AFMU is less efficiently extracted than 1X. The extraction process may add an extra error to the AFMU/1X ratios determined by HPLC. Not having to perform an extraction speeds up the analytical procedure considerably, and the total time needed for a phenotype determination using the CE method is around 20 min. Because of the innocuous nature of the probe drug, the fact that the test is non-invasive, and the speed of the analysis, this method should prove suitable for large-scale phenotyping studies.

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