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# Routine analysis of amphetamine class drugs as their naphthaquinone derivatives in human urine by high-performance liquid chromatography

Dinesh Talwar[a,*], Ian D Watson[b], Mike J Stewart[c]

[a]*Department of Clinical Biochemistry, Royal Infirmary, Glasgow, UK*
[b]*Department of Clinical Biochemistry, Aintree Hospitals, Liverpool, UK*
[c]*Department of Chemical Pathology, University Witwatersrand Medical School, Johannesburg, South Africa*

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**Abstract**

We describe a simple HPLC method which is suitable for the routine confirmation of immunoassay positive amphetamine urine samples. The precolumn derivisation method employing sodium naphthaquinone-4-sulphonate was found to have adequate sensitivity, selectivity and precision for the measurement of amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA), and 3,4-methylenedioxyethylamphetamine (MDEA) at 500 μg/l cutoff level for confirmatory analysis of amphetamines in urine. The specificity of the method is enhanced by detecting the peaks at two different wavelengths. The ratios of the peak heights measured at the two wavelengths were different for each of the 5 amphetamines analysed. There was no interference from other phenylethylamine analogues that are commonly found in ''over the counter'' preparations. The HPLC method is compared to a commercial TLC system for detecting amphetamines in urine of drug abusers attending drug rehabilitation programmes. The HPLC confirmatory method described is a viable alternative to GC or to the more complex and costly GC–MS techniques for confirming amphetamine, methamphetamine, MDMA, MDA and MDEA in urine of drug abusers especially when used in a clinical care setting © 1999 Elsevier Science B.V. All rights reserved.

*Keywords:* Amphetamines; Phenylethylamines; Naphthaquinone-4-sulphonate

## 1. Introduction

In the last decade, the abuse and recreational use of amphetamine and amphetamine analogues such as methamphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyethylamphetamine (MDEA) has increased dramatically in the UK and in other European countries.

Analysis of these amphetamines in urine is difficult due to the lack of specificity and/or sensitivity of the commonly used analytical methods. Preliminary testing of these drugs in urine is usually by immunoassay techniques which have adequate sensitivity but suffer from interference from chemically related phenylethylamines such as phenylpropanolamine, ephedrine and pseudoephedrine which are easily available in ''over the counter'' preparations

*Corresponding author. Tel.: +44-141-211-5178; fax: +44-141-553-1703.

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[1–3]. Some anorectics such as phenteramine and phenmetrazene can also produce a positive response to the amphetamine immunoassay [2].

The confirmation and identification of amphetamines (amphetamine, methamphetamine, MDMA, MDA and MDEA) in urine is usually by GC–MS or GC techniques. GC–MS is a sensitive and powerful technique for the positive identification of amphetamines in urine and the method of choice for laboratories involved in medico–legal work. These amines are usually confirmed with selective ion monitoring after derivatisation with either heptofluorobutyric anhydride or 4-carbethoxyhexafluorobutyryl chloride [4] or by using the technique of chemical ionisation [5,6]. However in the European Union (EU), unlike America, most of the clinical laboratories use urine drug analysis in the follow-up of drug addicts under clinical care, i.e., in the clinical care setting [7]. For many of these laboratories use of GC–MS for confirmation and identification of amphetamines in urine is often restricted by cost and expertise. A significant number of clinical laboratories in the EU use commercial TLC systems for the confirmation and identification of amphetamines in immunoassay positive urines [7]. TLC is both insensitive and relatively nonspecific due to the close migration of other phenylethylamines [8–10] although the Toxi Lab ''Sympathomimetic Differentiation'' TLC procedure has substantially improved the level of confidence in detecting amphetamine and its derivatives by TLC [11]. GC analysis requires derivatisation and use of electron capture detector (ECD) or nitrogen phosphorous detectors (NPD) to achieve the necessary sensitivity and specificity [12,13]. Although GC-NPD has adequate sensitivity, there may be potential interference from other drugs and analytes present in urine which may have GC retention times similar to that of the illicit drugs. Whilst this is also true of HPLC techniques especially when using a UV detector, the selectivity of HPLC methods can be enhanced if the analyte of interest can be selectively derivatised and the derivative has an absorption maxima at two different wavelengths. Measuring the peaks at two different wavelengths or monitoring their UV–visible spectra using a diode array can then enhance identification certainty.

HPLC has not been widely used to analyse amphetamines in urine because of their low and nonspecific UV absorptivities and low natural fluorescence. However several HPLC methods employing both normal- and reversed-phase modes and a variety of derivatising agents for UV, fluorescence and chemiluminescence detection of amphetamine and methamphetamine in urine have been described. Although the fluorescence and chemiluminescence techniques are highly sensitive for detection of amphetamine and methamphetamine in urine [14–18], chemiluminescent methods require complex postcolumn detection procedures [14,15]. For fluorescence detection, both on-line [16,17] and off-line [14,18] derivatisation procedures have been employed. However on-line derivatisation procedures require column switching equipment which is not readily available in clinical chemistry laboratories. Other methods have used precolumn derivatisation with naphthaquinone-4-sulphonate (NQS) [19–23] and spectrometric detection of the naphthaquinone derivatives of amphetamine and methamphetamine. Primary and secondary amino groups of amphetamines react with NQS in alkaline solution to form highly coloured compounds (Fig. 1) which can be determined colourimetrically [24]. Absorbance maxima occur in the visible region (450 nm) and in the UV region (260 nm). Derivatisation of amphetamine and methamphetamine with NQS results in a 25-fold increase in sensitivity in the visible range which is adequate for the microdetermination of these amines in urine [19,20]. Despite this only a few HPLC methods have been proposed in the literature for the detection of amphetamines in urine using derivatisation with NQS and HPLC separation. The naphthaquinone derivatives of amphetamines are usually separated by normal-phase HPLC [19–21].

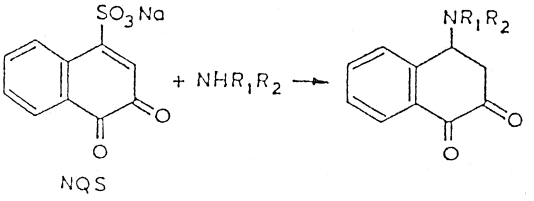


Fig. 1. Reaction of primary and secondary amino groups of amphetamines with 1,2-naphthaquinone-4-sulphonate in alkaline solution to form highly coloured compounds which can be determined colourimetrically. Absorption maxima occurs in the UV region (260 nm) and in the visible region (450 nm).

Reversed-phase is not normally used because of the long run time under isocratic conditions [22,23].

HPLC methods employing NQS as a derivatising agent are relatively simple and have adequate sensitivity (5 ng on column) which makes them suitable for the routine confirmation of positive screens detected using immunoassay. However none of these HPLC methods have been developed for the simultaneous analysis of other amphetamines that are abused extensively such as MDMA, MDA and MDEA. Recently Bogusz et al. reported an HPLC method for the simultaneous detection of these amines in urine as their phenylisothiocyanate derivatives using a diode array detection system [25]. The use of diode array enhanced the specificity of the method by monitoring the UV spectra of the derivatives of amphetamine and its analogues.

We describe an alternative approach to the identification and quantitation of amphetamines in urine based on their derivatisation with NQS. A normal-phase HPLC method was developed which was suitable for the routine confirmation of immunoassay positive amphetamine urine samples obtained from clients of drug rehabilitation programmes. The method requires a small urine volume (3 ml) and has adequate sensitivity, selectivity and precision for the detection of amphetamine, methamphetamine, MDMA, MDA and MDEA at the National Institute on Drug Abuse (NIDA now called Substance Abuse and Mental Health Services Association) cutoff level for confirmatory analysis of amphetamine and methamphetamine [26]. The selectivity of the method was enhanced by monitoring the naphthaquinone derivatives at two different wavelengths simultaneously, one in the UV region and the other in the visible region. The ratio of the peak heights at the two wavelengths were different for each of the amphetamine derivatives analysed.

## 2. Experimental

### 2.1. Materials

The phenylethylamines: amphetamine, methamphetamine, MDMA, MDA and MDEA, ephedrine, pseudoephedrine, phenylpropanolamine and dimethylamine (internal standard) were obtained from Sigma (Poole, UK). Stock standards (1 g/l) were prepared in deionised water.

Urine specimens were from drug addicts attending rehabilitation clinics, drug-free residential facilities and psychiatric units. Drug-free urine and Drugs of Abuse urine control were obtained from DPC (Diagnostics Products Corporation). The Drugs of Abuse urine control containing ten drugs and metabolites, was free of amphetamines and was used as ''blank'' urine for specificity studies. An aliquot of drug-free urine was spiked with amphetamine, methamphetamine, MDMA and MDA to obtain a combined urine control containing 5 mg/l of each of these amphetamines. Since the HPLC method was initially set up to detect amphetamine, methamphetamine, MDMA and MDA in urine, analytical studies on MDEA were performed separately at a latter stage by spiking drug-free urine with MDEA to generate a MDEA urine control containing 5 mg/l of the amine.

Sodium β-naphthaquinone-4-sulphonate (NQS) was obtained from Sigma. All other chemicals were of analytical reagent grade and obtained from BDH (Poole, UK).

### 2.2. Procedures

#### 2.2.1. Syva Emit ETS system

The Emit polyclonal and monoclonal amphetamine assays were performed using the ETS immunoassay system in accordance with the operators manual (Syva, Palo Alto, CA, USA).

#### 2.2.2. TLC

TLC analysis of amphetamines was carried out using the commercial Toxi Lab drug detection system according to the manufacturers instructions (Toxi Lab, Mercia, Guildford, UK).

#### 2.2.3. HPLC

*2.2.3.1. Apparatus*. HPLC analysis was performed using a Gilson pump, a Waters multiwavelength detector (Model 490) and a Rheodyne injector with a 20 μl loop.

*2.2.3.2. Extraction and derivatisation*. Urine samples (3 ml containing dimethylamine as internal standard 10 μl of 10 g/l solution) were extracted using commercial Toxi Lab A extraction tubes. After

centrifugation (2500 *g*, 2 min) the organic layer was transferred to conical tubes containing 20 μl of ethanolic HCl (6:1) and evaporated to dryness under nitrogen at 50°C. The residue was reconstituted with 0.5 ml of 8% sodium bicarbonate to which was added 0.5 ml of 0.5% NQS. The mixture was mixed and heated at 70°C for 20 min. After cooling the derivatives were extracted into 1 ml of chloroform. Under these conditions, reaction and extraction of amphetamines has been shown to proceed quantitatively [8].

*2.2.3.3. Chromatography and detection*. Twenty μl of extracted material was injected onto a 25 cm×4 mm ID silica HPLC column (5 micron, APEX, Jones Chromatography). The mobile phase was: hexane (145 ml); water saturated ethyl acetate (35 ml); chloroform (40 ml); ethanol (20 ml) which was pumped at a flow-rate of 1 ml/min. The Waters 490 detector is capable of monitoring four analog output channels simultaneously. Peaks were monitored at 260 nm (0.05 absorbance units full scale (AUFS)) and 450 nm (0.025 AUFS) on Channel 1 and Channel 2 respectively. Signals from these output channels were recorded using two chart recorders (JJ Instruments, Southampton, UK).

*2.2.3.4. Precision and accuracy studies*. The precision and accuracy of the assay was evaluated using dimethylamine as the internal standard. The combined urine control containing amphetamines (5 mg/l) was diluted 1:5, 1:10 and 1:20 to generate three urine controls containing 1000, 500 and 250 μg/l of amphetamine, methamphetamine, MDMA and MDA. Ten μl of internal standard (10 g/l) was added to these combined urine controls before extraction. Peak heights were measured at 450 nm. Precision and accuracy studies on MDEA were performed separately using the MDEA urine control (5 mg/l) which was diluted with drug-free urine to generate three control levels containing 1000, 500 and 250 μg/l of MDEA. These were then used for precision studies as described above.

*2.2.3.5. Identification and quantitation of amphetamines in urine*. Amphetamine, methamphetamine, MDMA, MDA and MDEA in urine samples were identified by both their capacity ratios ($k'$) and the ratio of their peak heights at 260 and 450 nm. The mean ratio for each of the amphetamines was calculated by analysing a combined urine control (containing 500 μg/l of amphetamine, methamphetamine, MDMA and MDA) and a MDEA urine control (containing 500 μg/l of MDEA) 17 times over a period of 16 weeks. Peak heights were measured simultaneously at 260 nm and at 450 nm. The amphetamines in urine were positively identified only if their peak height ratios were within two standard deviations (SD) of their means obtained using urine controls.

Urine concentrations of amphetamines were based on an extracted standard curve. Calibration curves were obtained for amphetamines spiked urines by plotting their peak heights ratio to the internal standards versus drug concentration. Peak heights for the calibration curve were measured manually at 450 nm. Two extracted urine standards (containing 500 and 1000 μg/l of each of the amphetamines) were run with every batch of samples. Amphetamines were quantified at 450 nm instead of 260 nm since the latter wavelength was found to be less specific. This was at the expense of sensitivity as UV detection was approximately two-times more sensitive than visible range detection.

*2.2.3.6. Specificity studies*. To assess whether there was interference from nonamphetamine phenylethylamine derivatives, ephedrine, pseudoephedrine, and phenylpropanolamine were added to drug-free urine which was then extracted and analysed by HPLC as described above. To check for any potential interferences from some of the other commonly abused drugs, Drugs of Abuse urine control was also extracted and analysed by HPLC. This urine control contained the following drugs and metabolites: benzoylecogonine (5 mg/l), codeine (3 mg/l), methadone (1 mg/l), methaqualone (0.75 mg/l), morphine (0.25 mg/l), morphine-3-glucuronide (2.75 mg/l), oxazepam (1 mg/l), phencyclidine (0.25 mg/l), secobarbital (2 mg/l) and tetrahydrocannabinol (0.25 mg/l).

### *2.3. Analysis of samples*

During a 3 month period 700 urine specimens were screened for amphetamines by the ETS immunoassay system. Urine specimens that were positive by both the polyclonal and monoclonal assays

were analysed by TLC Toxi Lab and also by HPLC. A urine specimen was considered positive by HPLC if the concentration of an amphetamine quantified with a calibration curve was greater than the upper threshold value of 500 μg/l. With every batch of test specimens analysed by HPLC, a combined urine control containing 500 μg/l of each of the amphetamines was used as an internal quality control (QC).

### 2.4. External QC

The performance of HPLC method was monitored by taking part in the UK National External Quality Assessment Scheme for Drugs of Abuse in Urine (UKNEQAS).

## 3. Results

### 3.1. Chromatography

Fig. 2 shows a ''blank'' urine and Fig. 3 shows separation of amphetamines prepared from spiked urine. The methamphetamine, MDMA, amphetamine, MDA and dimethylamine (internal standard) were easily separated and had capacity ratios ($k'$) of 0.87, 1.13, 1.27, 1.53 and 4.33 respectively. The chromatography was found to be highly reproducible once equilibrium with the water saturated ethyl acetate was established. The mobile phase employed by us was similar to that used by Endo et al. [19]. The water in the mobile phase ''caps'' the active

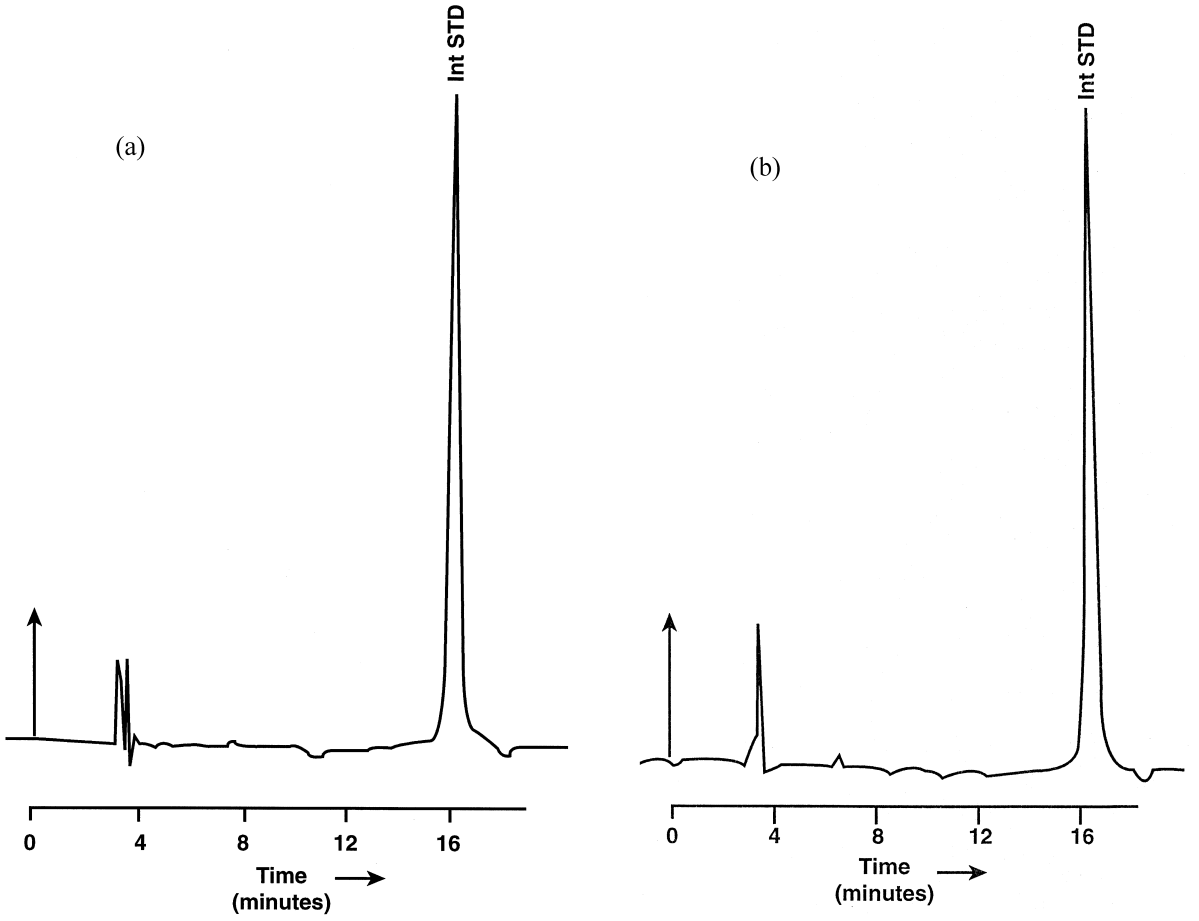


Fig. 2. Chromatogram of a ''blank'' urine. ''Blank'' urine was Drugs of Abuse urine control containing ten drugs and metabolites but free of any amphetamines. The chromatographic conditions are as described in the text. (a) Channel 1: detection at 260 nm (0.05 AUFS). (b) Channel 2: detection at 450 nm (0.025 AUFS).

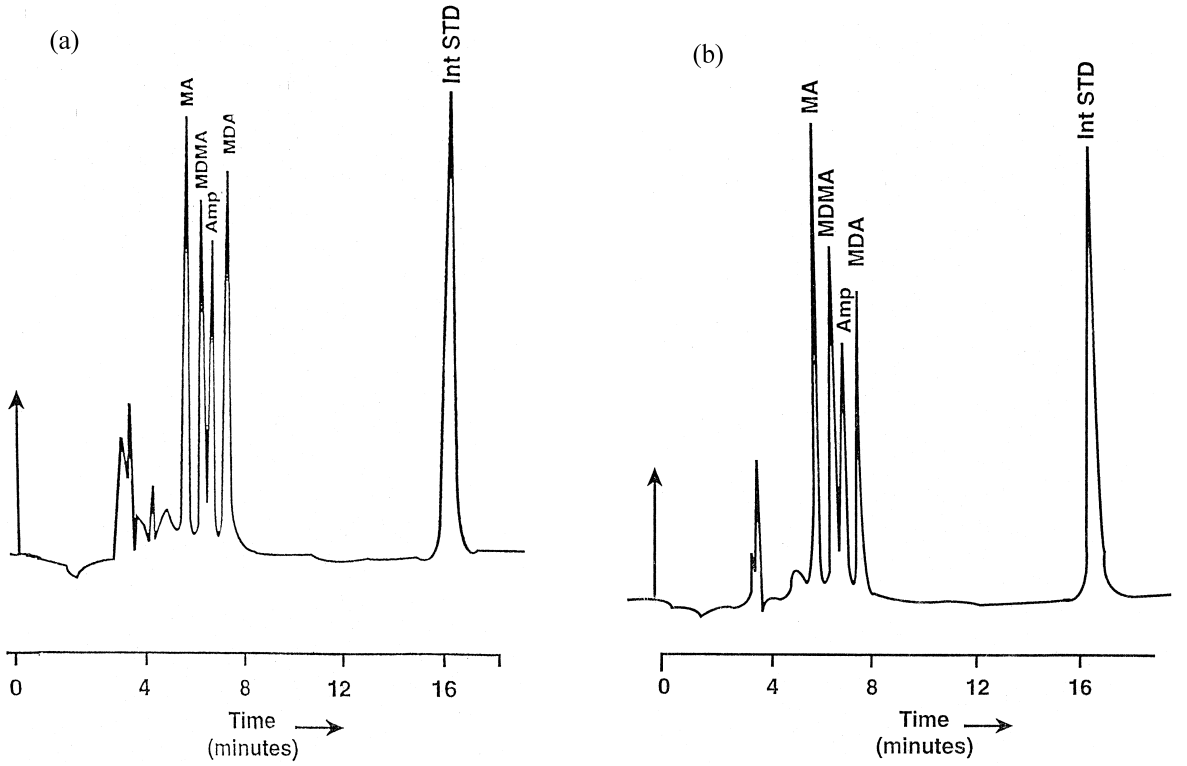


Fig. 3. Chromatogram of a combined urine control extract containing methamphetamine, MDMA, amphetamine and MDA each at a concentration of 500 μg/l. The combined urine control was prepared as described in the text. (a) Channel 1: detection at 260 nm (0.05 AUFS). (b) Channel 2: detection at 450 nm (0.025 AUFS).

silanol groups on the silica packing and as a result narrow and symmetrical peaks are produced. Fig. 4 shows the chromatogram obtained from the urine of a drug abuser who had admitted to have taken amphetamine Fig. 5 shows the chromatogram from the urine of a drug abuser who had admitted to taking ''ecstasy'' tablets (MDMA) and MDA.

The MDEA peak showed a near baseline separation from methamphetamine with $k'$ of 0.73 (Fig. 6). MDEA and methamphetamine have sufficiently different $k'$ and mean peak height ratio values to reliably distinguish the two amines in the unlikely event of a urine sample containing both the amines. Fig. 7 shows the chromatogram obtained from the urine of a drug addict who had taken ''eve'' (MDEA), amphetamine and MDA.

The mean ratio ($\pm 2$ SD) of the peak heights of MDEA, methamphetamine, MDMA, amphetamine, and MDA determined at 260 and 450 nm were 1.3 (0.10), 0.89 (0.11), 0.98 (0.08), 1.21 (0.10) and 1.25 (0.12) respectively. This ratio served as a check for peak purity and interference from coexisting peaks. The amphetamines in urine were positively identified only if their ratio was within two SD of the mean.

UV detection was approximately twofold more sensitive than visible range detection despite detection at 260 nm suffering from a noisier baseline compared to that at 450 nm.

### *3.2. Linearity and detection limits*

The calibration curves for all derivatised amphetamines in urine were linear upto at least 3500 μg/l at 450 nm. The linear relationship between the mean peak height ratio ($n=3$) of the target compounds to the internal standard and sample concentration were

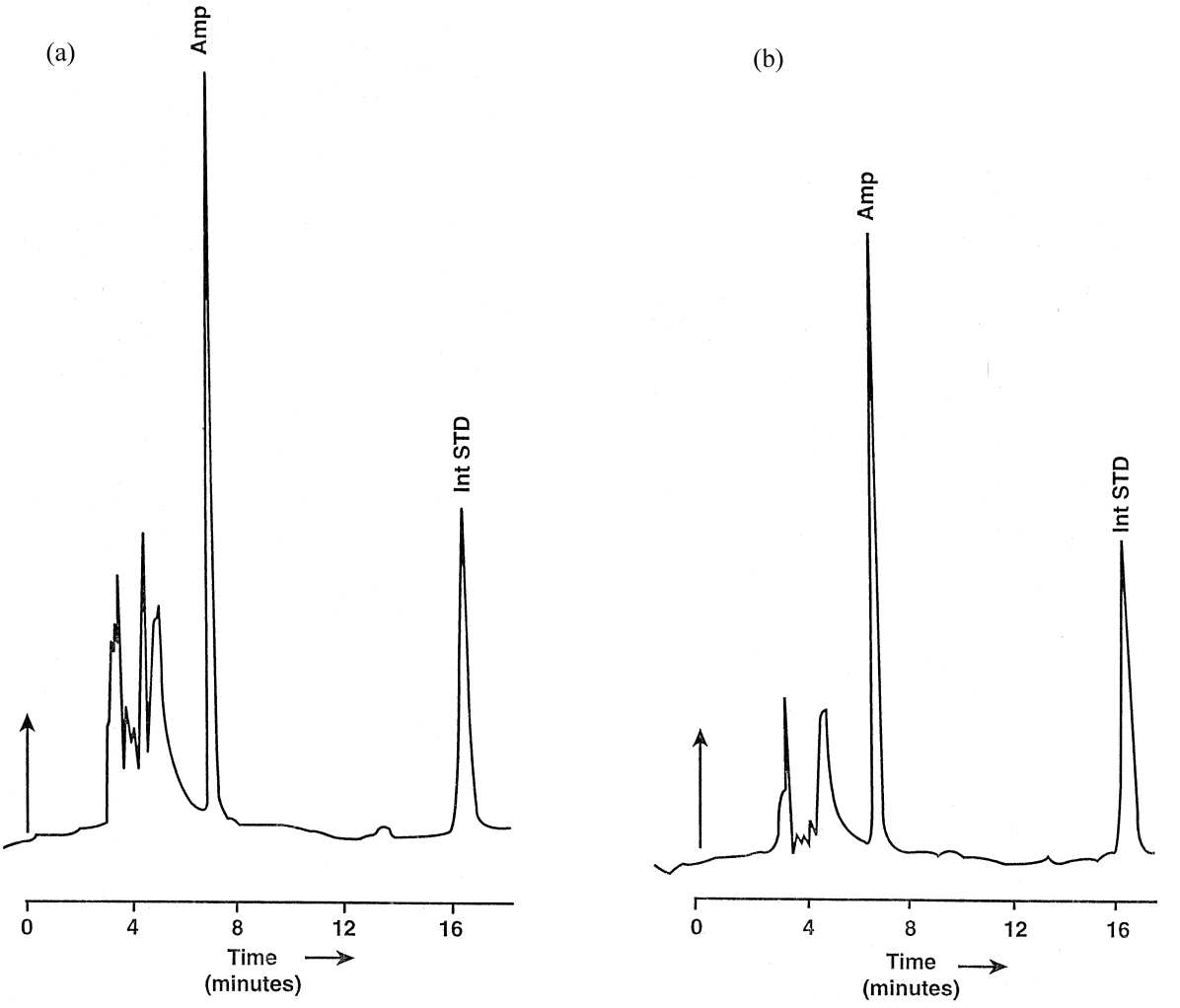


Fig. 4. Chromatogram of a urine extract from an amphetamine user. Amphetamine was present at a concentration of approximately 2000 μg/l. (a) Channel 1: detection at 260 nm (0.05 AUFS). (b) Channel 2: detection at 450 nm (0.025 AUFS).

obtained in the concentration range of 250–3500 μg/l. The regression equation for the calibration curve of amphetamines was as follows: MDEA, $y = (0.89 \cdot 10^{-3})x - 0.012$, $(r = 1.0)$; methamphetamine, $y = (1.18 \cdot 10^{-3})x - 0.013$, $(r = 1.0)$; MDMA, $y = (0.97 \cdot 10^{-3})x - 0.013$, $(r = 0.99)$; amphetamine, $y = (1.03 \cdot 10^{-3})x - 0.009$, $(r = 1.0)$; MDA, $y = (0.72 \cdot 10^{-3})x - 0.08$, $(r = 0.99)$ where $y$ is the peak height ratio of the target compound to internal standard and $x$ is the concentration in μg/l.

The limits of detection at 450 nm and 260 nm respectively under the conditions specified were as follows: methamphetamine, 90 and 65 μg/l; MDMA, 120 and 75 μg/l; amphetamine, 105 and 60 μg/l; MDA, 135 and 75 μg/l and MDEA, 120 and 65 μg/l. Detection limit was defined as the lowest concentration of the analyte in spiked urine that could be detected from zero with 97% confidence $(n = 10)$.

The limits of quantification (LOQ) of amphetamines in urine at 450 and 260 nm respectively were as follows: methamphetamine, 210 and 140 μg/l;

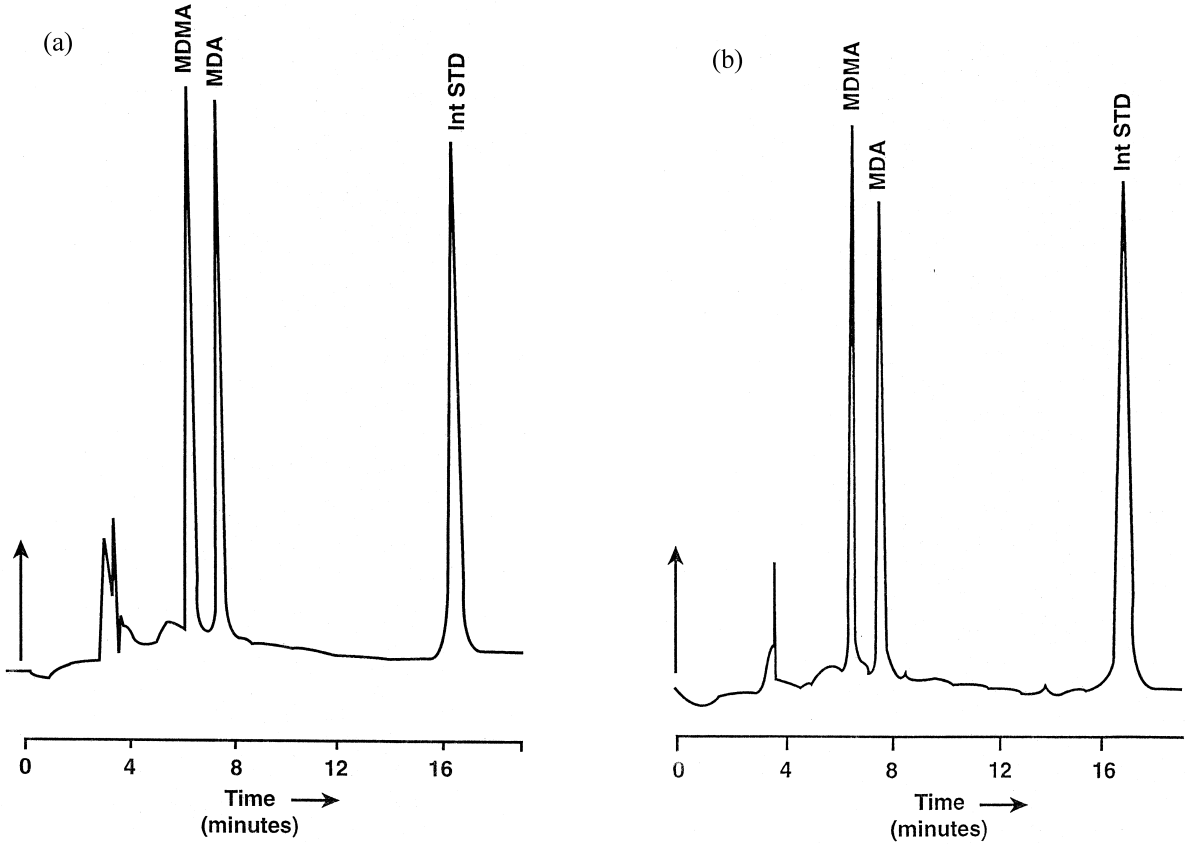


Fig. 5. Chromatogram of urine extract from a drug addict using both MDMA and MDA. The concentration of MDMA and MDA in the urine sample was approximately 750 μg/l and 700 μg/l respectively. (a) Channel 1: detection at 260 nm (0.05 AUFS). (b) Channel 2: detection at 450 nm (0.025 AUFS).

MDMA, 250 and 145 μg/l; amphetamine, 230 and 115 μg/l; MDA, 330 and 155 μg/l; MDEA, 270 and 125 μg/l. LOQ was defined as ten-times the signal-to-noise ratio.

### *3.3. Accuracy and precision studies*

Accuracy data is presented in Table 1. The repeatability and intermediate imprecision is shown in Table 2. Accuracy and repeatability data was obtained from spiked urine controls analysed 20 times on the same day. Intermediate imprecision was obtained by analysing spiked urine controls over a period of 16 weeks. The HPLC method has acceptable accuracy (<5% bias) and precision at the cutoff level for confirmatory analysis (500 μg/l).

### *3.4. Specificity studies*

There was no interference from ephedrine, pseudoephedrine or phenylpropanolamine. The capacity ratios ($k'$) for ephedrine and phenylpropanolamine were 6.24 and 7.4 respectively. Pseudoephedrine did not elute from the column under the chromatographic conditions described. No interference was observed from any drugs that were present in the Drugs of Abuse urine control (Fig. 1). Urine from patients who were prescribed tricyclic antidepressants was also assessed for potential interference since some of these drugs and their metabolites contain a secondary amino group. No interference was observed from tricyclic antidepressants (amitriptyline, imipramine) or their metabolites (nor-

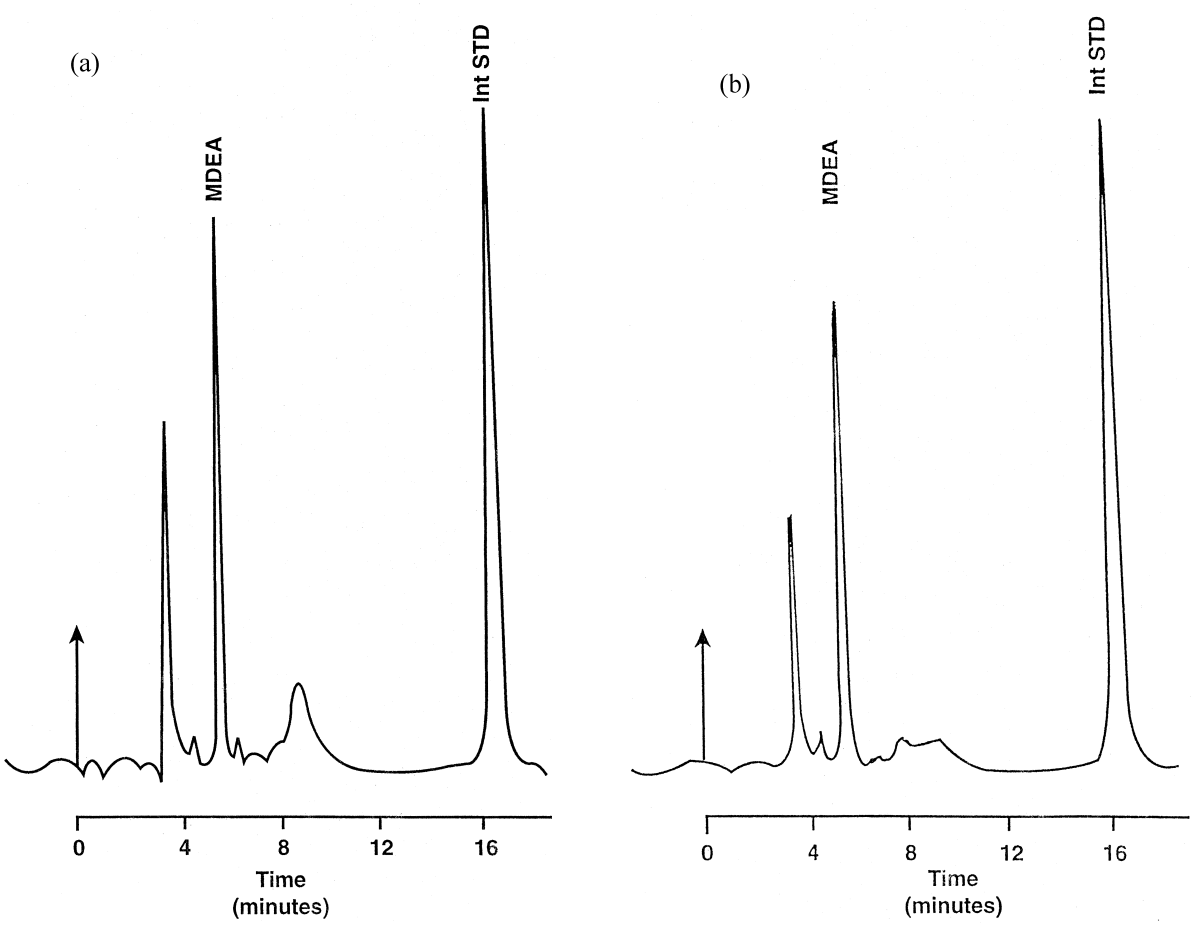


Fig. 6. Chromatogram of MDEA urine control containing 500 μg/l MDEA. This urine control was prepared by spiking drug-free urine with MDEA. (a) Channel 1: detection at 260 nm (0.05 AUFS). (b) Channel 2: detection at 450 nm (0.025 AUFS).

triptyline, desimipramine) under the chromatographic conditions specified.

### *3.5. Recovery studies*

Recovery studies were performed on a drug-free urine sample that was spiked with each of the amphetamines at a concentration of 1000 μg/l and then extracted and analysed as described above for samples. The mean recovery of drugs from spiked urine was 78% ($n=5$). Although the mean recovery of exogenously added amphetamines was never 100% with Toxi Lab extraction tubes, the use of an internal standard was the best way to correct for these differences in recovery.

### *3.6. Stability of naphthaquinone derivatives of amphetamines*

The extracted naphthaquinone derivatives of amphetamines were stable for at least 24 h at room temperature.

### *3.7. Comparison of methods*

Of the 700 urine samples screened by immunoassay, 56 were positive. Of these 56 positives, 43 were confirmed positive by Toxi Lab and 47 by HPLC. All urine samples that were confirmed positive by Toxi Lab had an amphetamine concentration greater than 1000 μg/l by HPLC. Seven samples

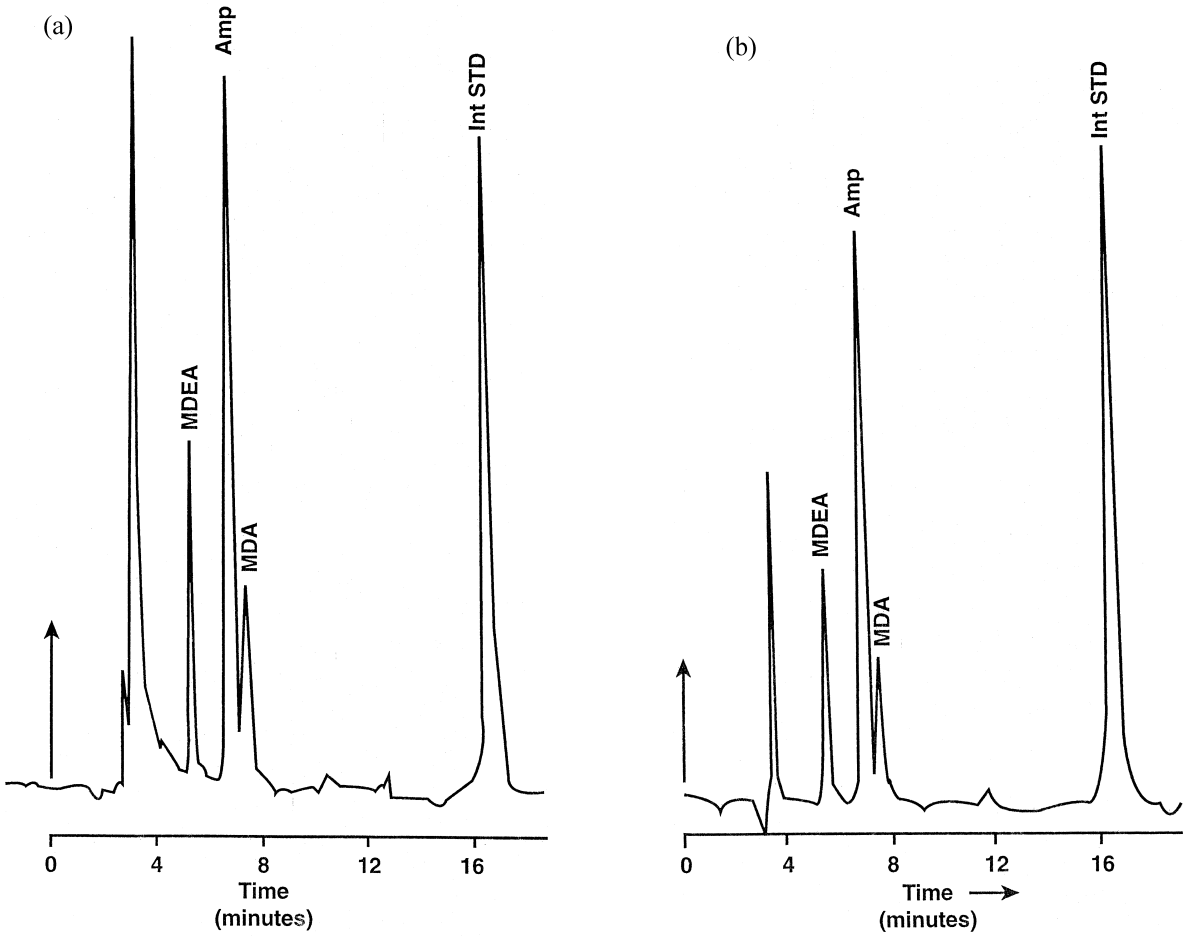


Fig. 7. Chromatogram of urine extract from a drug addict abusing MDEA, amphetamine and MDA. The urine was diluted fivefold prior to extraction. Concentration of MDEA, amphetamine and MDA in the neat urine sample was approximately 1500 μg/l, 4000 μg/l and 850 μg/l respectively. (a) Channel 1: detection at 260 nm (0.05 AUFS). (b) Channel 2: detection at 450 nm (0.025 AUFS).

Table 1
Concentration (μg/l) of amphetamines observed (at 450 nm) in spiked urine controls via calibration graphs; each urine control was run twenty times ($n=20$)[a]

| Concentration added | Observed concentration [Mean (SD)] | | | | |
|---|---|---|---|---|---|
| | MDEA | MA | MDMA | AMP | MDA |
| 250 | 265 (23) | 236 (19) | 261 (22) | 240 (18) | 263 (18) |
| 500 | 518 (38) | 481 (46) | 524 (27) | 520 (48) | 479 (43) |
| 1000 | 973 (68) | 970 (57) | 963 (65) | 1030 (59) | 979 (51) |

[a] MA = methamphetamine; AMP = amphetamine.

Table 2
Precision of measurements of amphetamines in urine[a]

| Concentration (μg/l) | % MA | % MDMA | % AMP | % MDA | % MDEA |
|---|---|---|---|---|---|
| Repeatability ($n=20$) | | | | | |
| 250 | 8 | 8.6 | 7.7 | 7.1 | 8.9 |
| 500 | 9.6 | 5.1 | 9.3 | 9.0 | 7.4 |
| 1000 | 5.9 | 6.7 | 5.8 | 5.2 | 6.9 |
| Intermediate ($n=17$) | | | | | |
| 250 | 8.8 | 9.3 | 7.9 | 7.7 | 9.7 |
| 500 | 7.5 | 8.1 | 7.1 | 7.0 | 8.3 |
| 1000 | 6.6 | 7.3 | 6.3 | 6.9 | 7.7 |

[a] MA = methamphetamine; AMP = amphetamine.

which had an amphetamine concentration between 500 and 1000 μg/l by HPLC were not picked up by Toxi Lab. Three urine samples which contained high concentrations of ephedrine gave positive results with TLC but were negative for amphetamines when analysed by HPLC. Both Toxi Lab and HPLC picked up MDMA and MDA in the urine samples from drug abusers who admitted taking MDMA and/or MDA.

Five external QC samples (QC1–5, UKNEQAS for Drugs of Abuse) were also analysed by the two methods. QC1 contained both methamphetamine (700 μg/l) and amphetamine (700 μg/l), QC2 was spiked with amphetamine alone (1500 μg/l) and QC3 was spiked with MDA (700 μg/l) and amphetamine (1000 μg/l). QC4 and QC5 were negative for amphetamines. One of the QC samples (QC5) containing high concentrations of ephedrine (5000 μg/l) and phenylpropanolamine (5000 μg/l) gave a false positive for amphetamine by Toxi Lab, but was correctly identified by HPLC. All five external QC samples were correctly identified by HPLC.

## 4. Discussion

We found the NQS derivatisation of amphetamines and their subsequent analysis by HPLC, to be reliable, selective and sufficiently sensitive for the quantitative analysis of these amines in urine of drug addicts. Phenylethylamine analogues that are commonly found in ''over the counter'' preparations do not interfere with the HPLC method. At the cutoff level for confirmatory analysis (500 μg/l), the HPLC method has acceptable precision and sensitivity. The limit of detection in the visible range is comparable to that obtained by GC-NPD (5 ng on column.).

When the HPLC method was first set up in 1993, there was a lack of agreement on the cutoff concentrations used by clinical laboratories in the EU for the confirmation of amphetamines in urine. We selected a cutoff concentration of 500 μg/l to conform with NIDA guidelines [26] which were the only specific regulations on cutoff concentrations for methamphetamine and amphetamine developed at that time. However recently the European Toxicology Group of Experts have defined a lower cut off concentration (200 μg/l) for confirmation of amphetamine, methamphetamine MDMA, MDA and MDEA in urine [27]. At this lower cutoff value, the HPLC method as described would have inadequate sensitivity to reliably measure amphetamines in urine. However the method has adequate sensitivity if amphetamines are quantified using 260 nm wavelength instead of 450 nm. The LOQ at 260 nm is approximately two-times less than at 450 nm. In addition our preliminary work suggests that the LOQ at 450 nm can be lowered by 2.5-fold by increasing the injection volume to 50 μl without adversely affecting the column performance or signal-to-noise ratio.

HPLC is possibly less selective than GC in that the chromatography of polar and nonvolatile compounds is not excluded. As a consequence the potential for interference by extraneous compounds is considerable especially when nonselective UV detection is used. However the detection of amphetamines as their naphthaquinone derivatives at two different wavelengths enhances the specificity of the assay and increases the possibility of accurate identification of these drugs in urine. The ratios of peak heights measured at 260 and 450 nm were different for each of the amphetamines analysed. Derivatisation coupled with dual wavelength monitoring makes this HPLC method a selective and sensitive technique for analysing amphetamines in urine. It is therefore a viable alternative to GC-NPD or to the more costly and complex GC–MS techniques for confirming amphetamine, methamphetamine, MDMA, MDA and MDEA in urine of drug abusers particularly when used in the clinical care setting.

Recently Bogusz et al. reported an HPLC method for the determination of amphetamines in urine as their phenylisothiocyanate derivatives using UV spectrometry as diode array detection [25]. The detection limit of their method is comparable to the NQS procedure for the determination of amphetamines in urine (100 μg/l). The advantage of using a diode array detection system is its ability to monitor the spectra of the derivatives thereby enhancing the identification certainty. However the UV spectra of phenylisothiocyanate derivatives of MDMA and MDEA were practically identical with maxima of 236–240 nm, whilst the other amphetamines showed only slightly different spectra with

maxima of 245–250 nm [24]. Since the naphthaquinone derivatives of amphetamines show an absorption maxima both in the UV and visible regions, monitoring the UV–visible spectra of the derivatives with a diode array may potentially further improve the specificity of the NQS procedure.

The NQS procedure as described here for determination of amphetamines in urine is less sensitive than chemiluminescence or fluorescent techniques and since it requires reextraction of NQS derivatives, it is more labour intensive and time consuming than the latter techniques. A rapid direct fluorescent HPLC method has been proposed by Sadeghipour and Veuthey [28] for the determination of MDEA, MDMA and MDA in illicit tablets and serum. However this method cannot be used to detect methamphetamine or amphetamine since these amines have low natural fluorescence. Recently Campins et. al. [21] have developed a rapid method for the extraction and derivatisation of amphetamines in urine with NQS whereby sample clean-up and derivatisation is achieved simultaneously on solid-phase cartridges. The analysis time of this procedure is similar to that of chemiluminescent and fluorescent methods (approximately 15 min).

Results obtained by HPLC and Toxi Lab generally showed good agreement. In our experience with the two techniques, the Toxi Lab system was far less sensitive and specific than the HPLC method.

The HPLC method described in this paper is simple and rapid requiring equipment commonly found in clinical chemistry laboratories. The extraction procedure using Toxi Lab A extraction tubes was found to be highly efficient. Only 3 ml of urine is required to obtain adequate sensitivity which is similar to that reported by others using solid-phase extraction [21–23]. The method has been in routine use in our laboratory for over 5 years for the confirmation of over 4000 immunoassay positive amphetamine urine samples During this period we have participated in the UKNEQAS programme for Drugs of Abuse where the HPLC method has a performance score of 100% for confirming amphetamines in urine. Using the HPLC method as a confirmatory method, the false positive rate obtained with immunoassay was approximately 15% over a period of 5 years.

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