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# Determination of urinary normetanephrine, metanephrine and 3-methoxytyramine by high-performance liquid chromatography with electrochemical detection: comparison between automated column-switching and manual dual-column sample purification methods

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ABSTRACT

This report describes a high-performance liquid chromatographic method with electrochemical detection for the simultaneous quantitation of urinary metanephrine, normetanephrine and 3-methoxytyramine. This method, which involves manual dual-column purification steps for the routine determination of urinary metanephrines, is compared with the previously used spectrophotometric Pisano method and an on-line sample preparation procedure, where the automated sequential trace enrichment (ASTED) apparatus is used for the column-switching procedure. In order to automate the metanephrine assay, the enrichment technique was evaluated against the reference chromatographic method. Bio-Rad urine controls gave coefficients of variation of less than 9% at all levels for the reference method. Values of less than 19% were found in the reference range with the enrichment method, and the recovery of 3-methoxytyramine was also too poor to be measured in normal concentrations. The linearity of both methods is sufficient to determine pathological levels of these biogenic amines. Future developments should be focused on decreasing the variation of between-day assays in an on-line, automated procedure.

## INTRODUCTION

In 1957 Armstrong *et al.* [1] described the metabolic pathways of the catecholamines [1]. Since then, the measurement of total urinary metanephrine (MN) has been found to be the most sensitive diagnostic index in pheochromocytoma and related neurogenic tumours, such as neuroblastoma and ganglioneuroma, and the best discriminator of essential hypertension and pheochromocytoma [2]. MNs closely reflect the amount of catecholamines (CAs) released into the circulation. Therefore, they increase not only in most patients with these carcinomas but also when physiological sources of CAs are chronically activated. Because MNs are excreted in both conjugated and unconjugated forms, they are routinely measured after acid hydrolysis or sulphatase pretreatment. Unlike CAs, total MN excretion is not significantly influenced by diet [3], and they are present in higher concentrations and are more stable than urine CAs.

Several processes, based on photometry [4], electrophoresis [5,6], thin-layer chromatography [7], fluorometry [8,9] high-performance liquid

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chromatography (HPLC) [10–14], gas chromatography [15,16], mass spectrometry [17,18] and radioimmunoassay [19] have been developed for the analysis of total urinary normetanephrine (NMN), MN and 3-methoxytyramine (3-MTA). Urinary MNs have generally been measured by a spectrophotometric method, but nowadays the HPLC method is available in many laboratories and can be regarded as the reference method as it is not affected by interference from drugs containing CA-like compounds. Kissinger *et al.* [20] reported first the use of an electrochemical detector with an HPLC system to analyse CAs at the picogram level [20]. CAs and their metabolites are ideally suited for electrochemical detection (ED), since their electrochemical reactions are very rapid. Therefore, strong signals are obtained even at low working electrode potentials, where the background current and noise are low, and where very few other substances interfere electrochemically [11,14,21–23].

The automated sequential trace enrichment (ASTED) method involves the direct injection of a sample. The coordination of the operations of the ASTED and HPLC system has been described previously [21]. Hydrolysed urine samples are loaded into the trace enrichment cartridge (TEC) where a strong cation-exchange precolumn is used for sample clean-up and isolation of analytes. The MNs are eluted from TEC by the mobile phase into the analytical column.

Reversed-phase (RP) chromatography with dual-column sample purification has turned out to be suitable for the separation of 3-O-methylated CAs [22]. The reference method includes a purification procedure in which the first clean-up step uses cation-exchange columns, from which the eluate passes directly into strong anion-exchange columns. After elution from the latter columns the samples are injected into the analytical column.

The isocratic separation of MNs used an RP column with an ion-pair (1-octane-sulphonic acid, Pic B-8) mobile phase. 4-Hydroxy-3-methoxybenzylamine hydrochloride is used as an internal standard to aid analyte identification and quantitation of the MNs.

This paper reports the validation of the present HPLC method with manual purificaton procedure used for routine determinations of urinary MNs in our laboratory, and compares the results with those from the on-line (ASTED) purification procedure. The HPLC conditions for the ASTED and reference method were identical and therefore any differences between their analytical performances would be due to the different sample preparation procedures used.

## EXPERIMENTAL

### *Instrumentation*

In the reference method, samples were analysed with a Model 200 SSI pump (State College, PA, USA) and a Coulochem Model 5100 coulometric detector equipped with an analytical cell (Model 5010) and a guard cell (Model 5020, ESA, Bedford, MA, USA). Samples were injected with a Model 655A-40 autosampler from Hitachi (Tokyo, Japan). Peak heights were recorded on a Hewlett-Packard Model 3393A integrator and 9114B disc drive (Avondale, PA, USA).

In the ASTED method, the enrichment apparatus (Gilson Medical Electronics, Villiers-le-Bel, France) was used instead of the autosampler, and Separon HEMA-BIO 300 SB (2-hydroxyethylmethacrylate sulphobutyl, 10 $\mu$m) from Tessek (Czechoslovakia and Denmark) was used as a precolumn.

### *Chromatographic conditions*

All separations were performed on a Nova-Pak $C_{18}$ column (150 mm × 3.9 mm I.D., 4 $\mu$m particle size) from Waters (Milford, MA, USA) protected by a precolumn filter with a frit (55-0068 from ESA). This analytical column yields efficient separation in 15 min in the reference method. The mobile phase was prepared by mixing 62.4 g (0.07 *M*) of sodium dihydrogenorthophosphate ($NaH_2PO_4 \cdot 2H_2O$) obtained from BDH. (Poole, UK), three 25-ml bottles of 1-octanesulphonic acid, Pic B-8 (Waters), equivalent to 2.5 m*M* as the final concentration, and 594 ml of acetonitrile (LiChrosolv for chromatography) in 6 l of water. The pH was adjusted to 3.5 ± 0.01

with orthophosphoric acid. The flow-rate was 0.8 ml/min. The assay was carried out at room temperature. The mobile phase was filtered through a 0.45-μm filter from Millipore (Bedford, MA, USA). The Coulochem detector potentials were initially set at +0.20, +0.45 and +0.90 V for detector 1, detector 2 and the guard cell, respectively.

Because samples of very low ionic strength are injected at low flow-rates with low back-pressure, very long column lifetimes are obtained.

*Chemicals and reagents*

Metanephrine hydrochloride and normetanephrine hydrochloride were obtained from Sigma (St. Louis, MO, USA), 3-methoxy-4-hydroxyphenylethylamine hydrochloride (3-methoxytyramine) was purchased from Calbiochem-Behring (La Jolla, CA, USA). 4-Hydroxy-3-methoxybenzylamine hydrochloride was from Pfalz & Bauer (Waterbury, CT, USA).

NMN, MN and 3-MTA stock solutions were prepared separately at a concentration of 4 mg/ml in 0.1 *M* HCl. All stock calibration standards were stable at −20°C for a year.

A working standard mixture containing, per litre, 100 mg each of NMN, MN, and 3-MTA was made in 0.1 *M* HCl by diluting 50 μl of each stock standard to 2 ml of 0.1 *M* HCl. The standards 1–5 were diluted (5–80 μl in 4 ml) with 0.1 *M* HCl.

In the reference method, 4-hydroxy-3-methoxybenzylamine hydrochloride, used as the internal standard, was prepared at a concentration of 0.32 mg/ml in 0.1 *M* HCl, and 50 μl (16 μg) were used per sample. A reagent for dilution was prepared by dissolving 40 g of diammonium pentaborate, $(NH_4)_2B_{10}O_{16} \cdot 8H_2O$, obtained from Sigma, and 1.11 g of EDTA from Merck (Titriplex III) in 1.0 l of water. The reagent for elution was 0.15 *M* ammonium acetate (pH 6.0) obtained from Merck. The cation-exchange resin (Bio-Rex 70, 50–100 mesh, sodium form) and the anion-exchange resin (AG 1-X4, 50–100 mesh, chloride form) were purchased from Bio-Rad Labs. (Richmond, CA, USA). Hydrochloric acid, sodium hydroxide and glacial acetic acid were all obtained from Merck.

In the ASTED method, the internal standard concentration was 1.6 mg in 15 ml of 10 m*M* $H_2SO_4$ and 25 μl (3 μg) were used per sample. A diammonium hydrogenorthophosphate buffer (0.5 *M*, pH 8.3) was prepared by dissolving 66 g of $(NH_4)_2HPO_4$ from Merck in 800 ml of water. After the pH was adjusted to 8.3 with orthophosphoric acid, the volume was made up to 1.0 l with water.

*Assay of urinary metanephrines*

*Reference method.* A 4-ml sample of acidified urine was adjusted to pH 0.9 with 1 *M* HCl and hydrolysed at 100°C for 30 min. After cooling, 50 μl of the internal standard (16 μg in 0.1 *M* HCL) and 5 ml of 3% ammonium pentaborate buffer containing 0.1% EDTA were added, and the pH was adjusted to 6.5 with 1 *M* NaOH. The mixture was applied to the cation-exchange columns, which were packed in our laboratory. Borate binds CAs, so they were not retained on these columns. After washing with 5 ml of water, MNs were eluted with 8 ml of 2 *M* ammonium hydroxide into the anion-exchange columns, which were also packed in our laboratory. After washing with 5 ml of water, the MNs were eluted with 3 ml of 0.15 *M* ammonium acetate (pH 6.0). An aliquot of 40 μl of the eluate was injected into the analytical column.

To prepare the ion-exchange columns, the cation-exchange resin was first washed with an excess of distilled water, and after that twice with two to three volumes of 3 *M* HCl, 3 *M* NaOH, 3 *M* acetic acid, 1 *M* ammonium acetate (pH 6.5) for at least 30 min each, with very slow magnetic stirring, and finally five times with 0.1 *M* ammonium acetate (pH 6.5). The final adjustment to pH 6.5 was made with 2 *M* ammonium hydroxide. The anion-exchange resin was first regenerated by washing with an excess of distilled water and after that four times with 1 *M* NaOH and 1 *M* acetic acid. The pH was 3.8 after the last wash. The columns were packed with resin suspensions in short polyethylene Bio-Rex columns (Bio-Rad) plugged with a frit. The resins may be reused after regeneration. The chromatographic analysis time was 14.5 min per sample.

*ASTED method.* ASTED operates on-line with

the HPLC system, so that chemical and physical treatments of samples are performed in coordination with the data processor according to the programme for urinary free CAs in this method. The programmed chromatographic analysis time was 29 min.

Urine was hydrolysed at 100°C for 30 min in 0.5 *M* $H_2SO_4$ (1:1) and 50 μl of the 0.5 *M* diammonium hydrogenorthophosphate buffer (pH 8.3) and 25 μl of the internal standard solution (1.6 mg in 15 ml of 10 m*M* $H_2SO_4$) were mixed with 200 μl of the hydrolysed sample. Then 200 μl of the mixture were loaded into the TEC. This was followed by 2 ml of 0.01 *M* diammonium hydrogenorthophosphate buffer (pH 8.3). The analytes retained on the TEC were eluted into the analytical column by the HPLC solvent. The TEC was then re-equilibrated with 200 μl of 1 *M* $H_2SO_4$ and 800 μl of 0.01 *M* diammonium hydrogenorthophosphate buffer (pH 8.3). A sample can be pretreated during chromatographic separation of the previous sample.

*Quantitation*

The MNs were quantified by the peak-height ratio method, with 4-hydroxy-3-methoxybenzylamine hydrochloride as the internal standard, the peaks being identified by their retention times. The calibration standards gave correlation coefficients of 0.998 or better with the reference method. The results are reported automatically in μ*M*. This value is multiplied manually by the total volume of urine collected during 24 h. The absence of interfering peaks is proved, if necessary, by using two different working electrode potentials and comparing the ratio of the responses with those of the standards.

*Urine samples*

Urine specimens were collected over 24 h in polyethylene bottles containing 10 ml of 6 *M* HCl as preservative. The pH of the sample must be below 3, or bacterial growth may occur. Aliquots were found to be stable at least for two weeks at 4°C. Acidified samples (pH 1) may be stored for at least a month without addition of EDTA or ascorbic acid.

*Quality control material*

Lyphochek Quantitative Urine Control Normal 1 and Abnormal 2 from Bio-Rad were used for within-run and between-run accuracy and precision studies. The control at the normal concentration level was injected after every five samples during an overnight run, and the control at the abnormal concentration level only once.

## RESULTS

*Correlation of the reference method with spectrophotometric Pisano and ASTED methods*

Fig. 1 shows the correlation between total MNs (NMN + MN) in urine, as determined by the reference HPLC and spectrophotometric Pisano methods. The equation for the relationship is $y = 0.804\,44 + 0.683\,31x$, $r^2 = 0.444$. Fig. 2a shows the correlation between the reference and ASTED methods for NMN. The equation for the relationship is $y = -5.5703e^{-2} + 1.0454x$, $r^2 = 0.971$. Fig 2b shows the correlation for MN. The equation for the relationship is $y = -0.102\,30 + 1.4310x$, $r^2 = 0.793$.

*Determination of the reference range of the reference method*

The reference range of the present HPLC method was determined with 40 urine samples from the laboratory personnel and their children. The results can be seen in Table I.

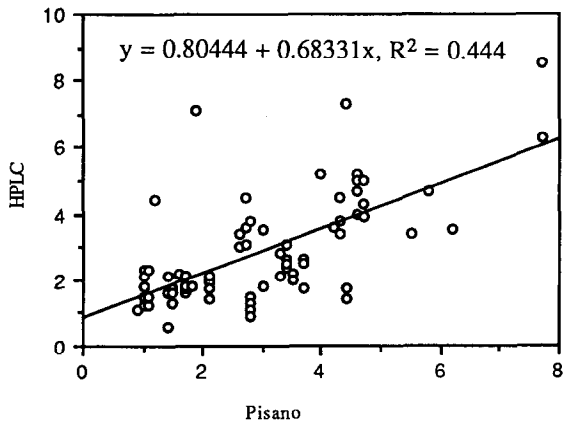


Fig. 1. Correlation between total MNs (NMN + MN) in urine, as determined with the reference HPLC method and spectrophotometric Pisano method.

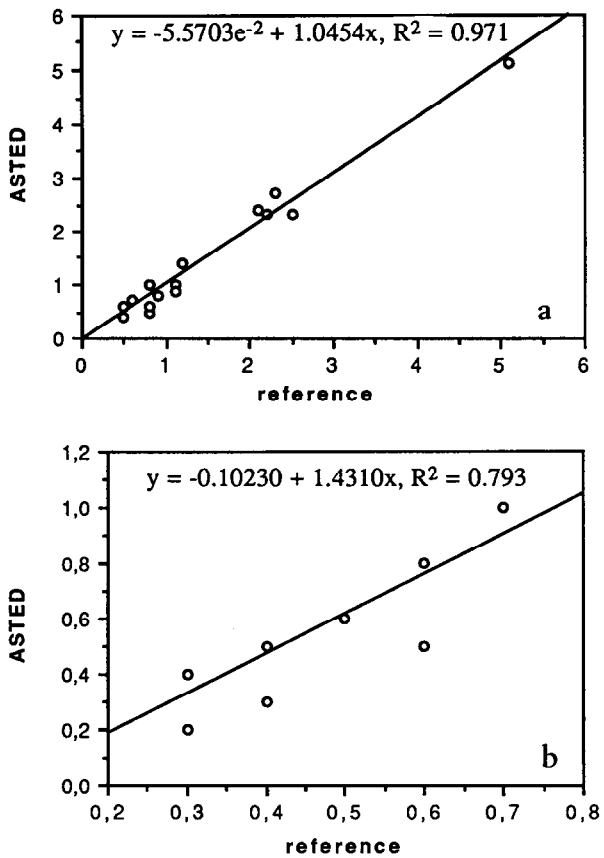


Fig. 2. Correlation between NMN (a) and MN (b) in urine, as determined with the reference HPLC method and the ASTED method.

*Chromatographic separation*

Fig. 3a shows a chromatogram of a standard corresponding to 104 μg of NMN, 107 μg of MN and 102 μg of 3-MTA. Fig. 3b shows a typical chromatogram of a hydrolysed urine sample from a healthy adult, and Fig. 3c that of urine from a patient with a pheochromocytoma assayed with the reference method. Fig. 4a and b shows corresponding chromatograms with the ASTED method, and Fig. 5 chromatograms of Bio-Rad urine control 2 (abnormal) by both methods.

*Precision*

The within-run imprecision of the reference method was estimated by assaying Lyphochek quality control materials at two concentrations. The between-run imprecision of the methods was estimated by assaying Lyphochek Quantitative Urine Control Normal with the reference method and Lyphochek Quantitative Urine Control Abnormal with the ASTED method. The results are shown in Table II.

*Sensitivity*

With the reference method we could quantitate concentrations of 40 μg/l NMN, 30 μg/l MN and 40 μg/l 3-MTA in actual urine samples, with coefficients of variation (C.V.) of 5–8% at a signal-to-noise ratio of 3.

*Analytical recovery*

The analytical recoveries were obtained by assaying five urine samples from different patients, supplemented with known concentrations of MNs, with the reference method ($n$ = 3). The added amounts ranged from 0.20 to 2.00 mg/l. The spiked urines were carried through the entire procedure and the concentrations were calculated against the standards. The recoveries of added MNs were 91 ± 3% (mean ± S.D.).

TABLE I

EXCRETION LIMITS FOR TOTAL MNs IN 24-h URINE OF NORMAL ADULTS AND CHILDREN

| Compound | $n$ | 2 years | | 16 years | | Adults (μmol) |
|---|---|---|---|---|---|---|
| | | μmol | μg/mg of creatinine | μmol | μg/mg of creatinine | |
| NMN | 40 | 1.8 | 2.6 | 2.0 | 0.5 | 4.2 |
| MN | 40 | 0.5 | 0.9 | 0.8 | 0.2 | 1.4 |
| 3-MTA | 40 | 0.6 | 0.7 | 1.2 | 0.3 | 2.3 |

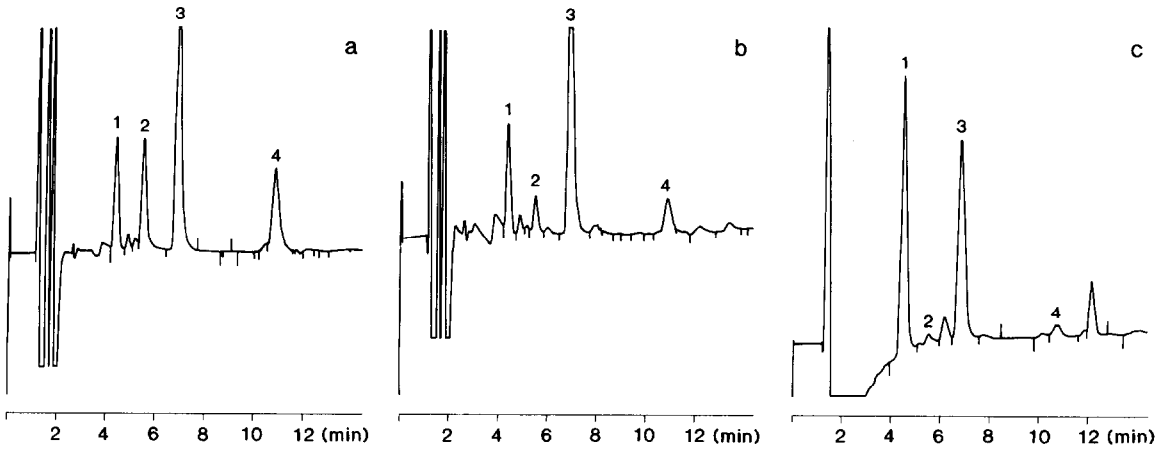


Fig. 3. Typical chromatograms of a standard, corresponding to 104, 107 and 102 μg of NMN, MN and 3-MTA, respectively (a), a urine sample from a healthy adult (b) and a urine sample from a patient with a pheochromocytoma (c), assayed with the reference method. Peaks: 1 = NMN; 2 = MN; 3 = internal standard; 4 = 3-MTA.

*Linearity*

The linearity of the reference HPLC method was tested in the expected range of concentrations to be assayed with standard addition to a pooled urine. The method turned out to be linear at least up to 70, 20 and 30 $\mu M$ for NMN, MN and 3-MTA, respectively, with a C.V., both within-run and between-run, of less than 9% at all levels.

DISCUSSION

Urinary MNs have been assayed routinely in our laboratory with the reported reference HPLC method for the past five years. Earlier, we analysed MNs by a spectrophotometric method that had some major limitations [4]. It could not distinguish between NMN and MN, and was not entirely specific for these compounds as is evident

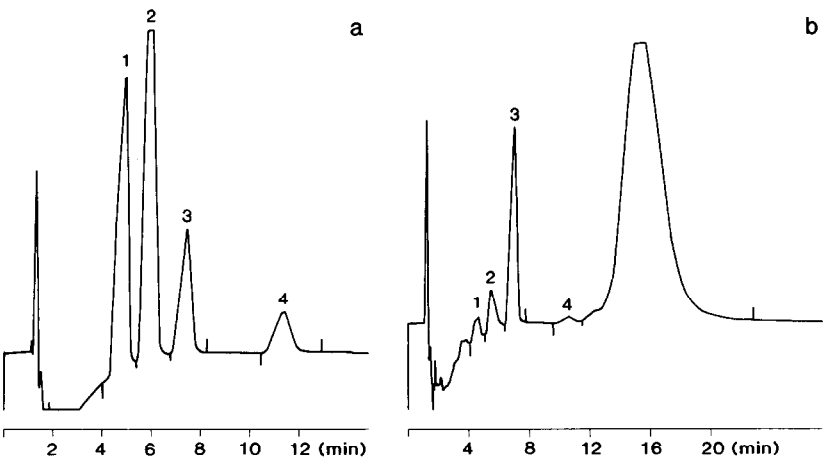


Fig. 4. Chromatograms of a standard, corresponding to 104, 107 and 102 μg of NMN, MN and 3-MTA, respectively (a) and a urine sample from a healthy adult (b) with the ASTED method. Peaks: 1 = NMN; 2 = MN; 3 = internal standard; 4 = 3-MTA.

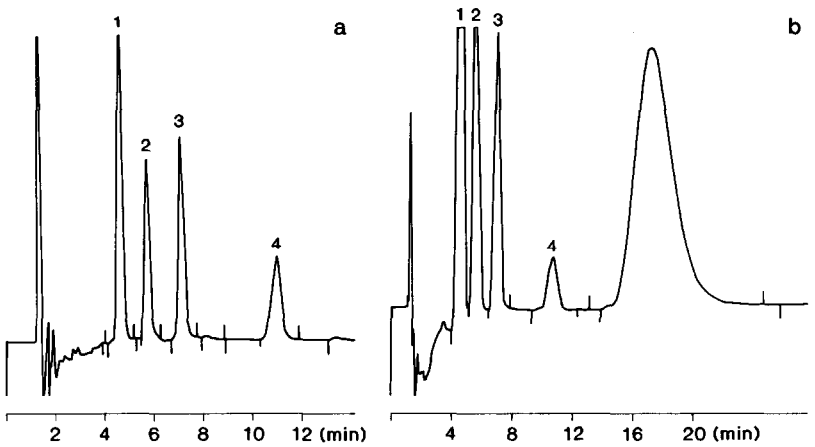


Fig. 5. Chromatograms of Bio-Rad urine control 2 (abnormal) with the reference method (a) and the ASTED method (b). Peaks: 1 = NMN; 2 = MN; 3 = internal standard; 4 = 3-MTA. Measured concentrations ($\mu M$) can be found in Table II.

from Fig. 1, which shows the correlation between the spectrophotometric Pisano and the reference methods. The spectrophotometric approach is still used in some laboratories because the extraction of MNs from urine for HPLC is relatively slow and tedious, requiring for example the adjustment of the pH to a fixed value and two ion-exchange purification steps. In Pisano's method the hydroxyl- and amino-containing side-chains are oxidatively cleaved by periodate after sample hydrolysis and isolation of the MNs by ion-exchange chromatography. Oxidation of both MN and NMN results in the formation of a common end-product, vanillin. Unfortunately, other endogenous compounds in urine absorb near the UV maximum of vanillin (347 nm). Vanillin absorption is monitored at 360 nm to minimize potential interferences, such as labetalol. Drugs such as α-methyldopa, isoprenaline, quinidine, propranolol, labetalol and tricyclic antidepressants, which also show fluorescent properties, may interfere in the traditional fluorescence procedure. When the highest analytical sensitivity and accuracy without interferences is needed, these traditional spectrophotometric and fluorometric methods for urinary CAs and their metab-

TABLE II

WITHIN- AND BETWEEN-RUN IMPRECISION OF THE REFERENCE AND ASTED METHODS

| Compound | Reference method | | | | ASTED method | | | |
|---|---|---|---|---|---|---|---|---|
| | $n$ | $x$ ($\mu M$) | C.V. (%) | | $n$ | $x$ ($\mu M$) | C.V. (%) | |
| | | | Within-run | Between-run | | | Within-run | Between-run |
| *Lyphochek normal* | | | | | | | | |
| NMN | 40 | 1.44 | 2.4 | 4.1 | 7 | 1.33 | 6.2 | – |
| MN | 40 | 0.48 | 2.9 | 8.7 | 7 | 0.37 | 5.2 | – |
| 3-MTA | 40 | 0.46 | 3.9 | 8.5 | 7 | 0.38 | – | – |
| *Lyphochek abnormal* | | | | | | | | |
| NMN | 40 | 5.51 | – | 5.7 | 5 | 5.57 | – | 10.2 |
| MN | 40 | 1.76 | – | 7.1 | 5 | 1.77 | – | 3.3 |
| 3-MTA | 40 | 1.87 | – | 7.5 | 5 | 2.03 | – | 18.6 |

olites are being replaced by chromatographic methods. Some compounds, such as the α-methyldopa metabolite α-methylnorepinephrine, may elute close to MN and NMN, depending on the chromatographic conditions. It is known that NMN coelutes with synephrine [23]. A few drugs have structures similar to that of MN. This group includes phenylephrine, terbutaline and orciprenaline. Labetalol may also interfere with MN analysis [24] and acetaminophen may falsely increase the estimation of NMN [25]. Varying amounts of acetonitrile and 1-octanesulphonic acid can be added to the mobile phase to eliminate possible interferences. ED with ion-pairing adaptations of RP chromatography are the most common methodologies used for urinary MN assays.

Bandi [26] has described a procedure based on Bio-Rad dual-column purification of MNs using RP-HPLC with coulometric detection. There have been some reports of the validity of the column-switching technique in the separation of CAs. An on-line clean-up procedure for urinary CAs has been described, which is based on the column-switching technique with a silica column loaded with phenylboric acid [27]. Green *et al.* [14] have reported an on-line system for urinary free CAs by HPLC using ASTED. Simultaneous quantitation of free CAs and O-methylated metabolites in urine by isocratic ion-pairing HPLC with amperometric detection after a one-step sample preparation on Bio-Rex 70 cation-exchange resin has also been reported [28]. However, in our laboratory, the major group of biogenic amines to be analysed is the total MNs. Trouvin and Billaud-Mesguich [12] and Wilson *et al.* [13] have eluted MNs from the cation-exchange column with dilute acid and injected the eluate without further purification into isocratic liquid chromatography equipment with ED.

From the reference ranges determined for the present reference HPLC method, it can be seen that the excretion of MNs increases with age, if expressed as μmol, and, decreases, if expressed as μg/mg of creatinine. In children, the primary reason for measuring urinary CA metabolites is for the diagnosis and follow-up treatment of the highly malignant tumour, neuroblastoma. Because it is difficult to collect reliable 24-h urine specimens from children under three years of age, it is more reliable to measure MNs in relation to urine creatinine (μg metabolite per mg creatinine).

In evaluating the ASTED procedure against the present manual sample purification procedure, the intra-assay variation was compared between assays. To check the reproducibility of the both methods, Bio-Rad urine controls 1 and 2 were analysed. The results (Table II) show acceptable precision and accuracy for all of the compounds with the reference method, and are in good agreement with the mean values given by Bio-Rad. The recovery of 3-MTA with the AST-ED method is so poor that it cannot be measured in the Lyphochek normal concentration level (Table II).

Fig. 5 shows chromatograms of Bio-Rad urine control 2 by both methods. This control has abnormally high concentrations of most of the endogenous biochemical compounds. The chromatogram obtained with the reference method shows no extra peaks, which indicates that the extraction procedure is satisfactory. In the chromatogram obtained with the ASTED method, one extra peak can be seen: its retention time varied from run to run. It is evidently caused by the column-switching programme, used with perhaps too short an analysis time. It was found that the automated procedure yielded an acceptable variation for NMN and MN in the reference concentrations, but 3-MTA could not be measured at the normal level. The inter-assay variation by the on-line purification procedure, however, was moderate in the abnormal concentration levels, with C.V. values ranging from 3 to 19%. It is probable that the optimum was not reached in the TEC conditions, although two solvents were used: 0.05% Triton X-100 (v/v) and 0.01 *M* diammonium hydrogenorthophosphate buffer (pH 8.3). Between the runs the TEC was washed and stored in water. It may be necessary to carry out TEC conditioning with an ion-pairing agent before the next run to ensure between-column reproducibility. Altering the elution volume and

the relative amounts of the organic solvent and the ion-pairing agent in the mobile phase could also improve the performance of the TEC. Higher concentrations of phosphate in the HPLC solvent could increase the elution of the analytes from the TEC.

The main requirement for ED is that of sufficient electrical conductivity of the mobile phase; this is satisfied by the use of aqueous solutions of ionized substances, possibly mixed with an organic modifier, such as methanol or acetonitrile. In RP systems acetonitrile is a stronger solvent than methanol, so it was used to give sharper elution fronts. In view of the polar character of MNs, both ion-exchangers and non-polar reversed phases can be used for their separation; these stationary phases are readily compatible with aqueous mobile phases. However, RP systems predominate and involve the use of aqueous buffer solutions or inorganic acids, usually containing ion-pairing agents, such as alkyl sulphonates, that form ion-pairs with protonated MNs and enhance the retention of MNs on non-polar stationary phases. Alteration of the chromatographic retention through modification of the ion-pairing agents, ionic strength, or organic components has been described previously [29]. Nevertheless, the recovery of 3-MTA by the ASTED method was poor, suggesting that the procedure for isocratic RP-HPLC was not optimal. The possibility that breakdown of 3-MTA might be occurring during the hydrolysis procedure in 0.5 *M* $H_2SO_4$ (1:1) was considered, but was eliminated by the fact that there were no differences in 3-MTA recoveries between these 1:1 hydrolysed samples and those hydrolysed and purified with the reference method and then analysed by the ASTED method. Although this on-line procedure is an apparently simple approach in practice, the development of suitable chromatographic conditions still presents problems.

The present study was undertaken because clinical laboratories, at the moment, are expected to improve quality while reducing cost. Automation is supposed to minimize variation, which is inherent in manual techniques, decrease labour, and improve laboratory throughput and the quality of the analytical output, at the same time. Commercial systems that offer complete automation of urinary metanephrine assays have only very recently begun to be developed.

In conclusion, the present HPLC procedure is still to be preferred as the routine method for accurate measurement of urinary NMN, MN and 3-MTA. However, it is reasonable to think that these measurements, if optimized properly, can be automated with high precision and recovery in the near future.

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REFERENCES

1 M. D. Armstrong, A. McMillan and K. N. F. Shaw, *Biochim. Biophys. Acta* 25 (1957) 422.
2 P. Moleman, *Clin. Chim. Acta*, 189 (1990) 19.
3 A. Krstulovic, *J. Chromatogr.*, 229 (1982) 1.
4 J. J. Pisano, *Clin. Chim. Acta*, 5 (1960) 406.
5 K. Yoshinaga, C. Stoh, N. Ishida, T. Sato and Y. Wada, *Nature (London)*, 191 (1961) 599.
6 R. L. Wolf, C. E. Gherman, J. D. Lauer, H. L. Fish and B. R. Levey, *Clin. Sci. Mol. Med.*, 45 (1973) 263.
7 J. M. C. Gutteridge, *Clin. Chim. Acta*, 21 (1968) 211.
8 Z. Kahane and P. Vestergaard, *Clin. Chim. Acta*, 25 (1969) 453.
9 H. Nohta, E. Yamaguchi, Y. Ohkura and H. Watanable, *J. Chromatogr.*, 467 (1989) 237.
10 R. E. Shoup and P. T. Kissinger, *Clin. Chem.*, 23 (1977) 1268.
11 J. Dutrieu and Y. A. Delmotte, *Fresenius' Z. Anal. Chem.*, 316 (1983) 505.
12 J. H. Trouvin and E. Billaud-Mesguich, *J. Liq. Chromatogr.*, 10 (1987) 731.
13 S. P. Wilson, D. L. Kamin and J. M. Feldman, *Clin. Chem.*, 31 (1988) 1093.
14 B. Green, J. D. H. Cooper and D. C. Turnell, *Ann. Clin. Biochem.*, 26 (1989) 361.
15 J. F. Van de Calseyde, R. H. J. Scholtis, N. A. Schmidt and C. J. J. A. Leyten, *Clin. Chim. Acta*, 32 (1971) 361.
16 L. M. Nelson, F. A. Bubb, P. M. Lax, M. W. Weg and M. Sandler, *Clin. Chim. Acta*, 92 (1979) 235.
17 F. A. J. Muskiet, C. G. Thomasson, A. M. Gerdin, D. C. Fremow-Ottevangers, G. T. Nagel and B. G. Wolters, *Clin. Chem.*, 25 (1979) 453.

18 C. Canfell, S. R. Binder and H. Khayam-Bashi, *Clin. Chem.*, 28 (1982) 25.
19 S. Oishi, M. Sasaki, M. Ohno and T. Sato, *J. Clin. Endocrinol. Metab.*, 67 (1988) 614.
20 P. T. Kissinger, R. M. Riggin and R. L. Alcorn, *Biomed. Med.*, 13 (1975) 299.
21 D. C. Turnell and J. D. H. Cooper, *J. Autom. Chem.*, 8 (1986) 151.
22 J. Jouve, N. Mariotte, C. Sureau and J. P. Muh, *J. Chromatogr.*, 274 (1983) 53.
23 T. Nagao and T. Tanimura, *Anal. Biochem.*, 171 (1988) 33.
24 J. M. Feldman, *J. Clin. Pharmacol.*, 27 (1987) 288.
25 S. P. Wilson, D. L. Kamin and J. M. Feldman, *Clin. Chem.*, 31 (1985) 1093.
26 Z. L. Bandi, *Clin. Chem.*, 35 (1989) 1138.
27 K. Kemper, E. Hagemeier, K.-S. Boos and E. Schlimmer, *J. Chromatogr.*, 336 (1984) 374.
28 Y. P. Chan and T. S. Siu, *J. Chromatogr.*, 459 (1988) 251.
29 P. Kontur, R. Dawson and A. Monjan, *J. Neurosci. Methods*, 11 (1984) 5.