Journal of Chromatography, 617 (1993) 181–189
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6902

Isotope dilution ammonia chemical ionization mass fragmentographic analysis of urinary 3-O-methylated catecholamine metabolites

Rapid sample clean-up by derivatization and extraction of lyophilized samples

Ido P. Kema*, Gert Meiborg, Gijs T. Nagel, Gerrie J. Stob and Frits A. J. Muskiet

Central Laboratory for Clinical Chemistry, University Hospital of Groningen, P.O. Box 30001, 9700 RB Groningen (Netherlands)

(First received December 27th, 1992; revised manuscript received April 27th, 1993)

ABSTRACT

We developed a method for simultaneous quantification of the urinary 3-O-methylated catecholamine metabolites 3-methoxytyramine, normetanephrine and metanephrine by stable isotope-dilution ammonia chemical ionization mass fragmentography. Prepurification of lyophilized samples was done by simultaneous deconjugation and pentafluoropropionylation, followed by extraction and rederivatization. Compared with our previously described method, based on acid hydrolysis, alkaline extraction, derivatization and electron-impact mass fragmentography, the present method was found to be less laborious, more sensitive and presumably more accurate. New urinary excretion values were established for apparently healthy adults. The present prepurification method may prove applicable for profiling of a variety of naturally occurring mono-, di- and polyamines in biological samples.

INTRODUCTION

Metabolism of the catecholamines dopamine (DA), norepinephrine (NE) and epinephrine (E) takes place by oxidative deamination (monoamine oxidase; EC 1.4.3.4), O-methylation (catechol-O-methyltransferase; EC 2.1.1.6), conjugation with sulphuric and glucuronic acids and combinations of these reactions (for review see ref. 1). Following neurotransmission processes catecholamines are primarily catabolized by monoamine oxidase, finally yielding acidic and alcoholic metabolites. Circulating catecholamines are primarily deactivated by catechol-O-methyltransferase, yielding the 3-O-methylated metabolites 3-methoxytyramine (3-MT; from DA), normetanephrine (NM; from NE) and metanephrine (M; from E). 4-O-Methylation is a minor process, accounting for about 5% of DA and 0.5% of NE and E metabolites [2]. The vast majority of catecholamine metabolites are excreted via the urine and are considered to derive from neurotransmission processes [1].

In clinical chemical laboratories quantification of catecholamines and their metabolites in body fluids is notably used in the diagnosis and follow-up of catecholamine-producing tumours (*i.e.*

* Corresponding author.

0378-4347/93/$06.00

neuroblastoma and phaeochromocytoma). Embryologically, both tumours derive from the neural crest. Phaeochromocytomas are predominantly located in the adrenal medulla. In a minority of cases they occur in and around the sympathetic ganglia, at locations that parallel the anatomical distribution of extra-adrenal chromaffin tissue, such as the organ of Zuckerkandl [3]. Phaeochromocytomas may be responsible for hypertension in approximately 0.1% of cases [3]. Characteristically they secrete NE, E or both, resulting in sustained or paroxysmal hypertension and increased urinary excretion of vanillylmandelic acid (VMA), 3-methoxy-4-hydroxyphenylethylene glycol (MHPG), NM, M, NE and E [4].

Extra-adrenal phaeochromocytomas, or paragangliomas, may merely produce and secrete NE. This is explained by their lack of phenylethanolamine-N-methyltransferase (PNMT; EC 2.1.1.28), which converts NE into E. PNMT in the adrenal medulla is induced by locally high concentrations of adrenal corticosteroids [5]. Low corticosteroid levels may prevent PNMT induction in extra-adrenal phaeochromocytomas [6]. Malignant phaeochromocytomas make up about 10% of cases. Additionally, increased urinary excretion of DA and its metabolites is regarded as a marker of malignancy [7,8]. Increased urinary excretion of N-methylepinephrine (NME) and its 3-O-methylated metabolite N-methylmetanephrine (MM) has been noted in several cases of adrenal phaeochromocytoma. Both compounds are believed to be secondary to E formation [9–11].

For the clinical chemical diagnosis and follow-up of patients with phaeochromocytoma measurements of plasma catecholamines [12,13], urinary catecholamines (notably free NE [14]), urinary acidic catecholamine metabolites (notably VMA [15]), and urinary total (sum of free and conjugated) metanephrines (NM and M [3,16]) have been used. Correct interpretation of plasma catecholamine concentrations requires cumbersome standardization of blood sampling to avoid stress-related artefacts. The need for protocolized blood sample collection hampers diagnosis of patients with paroxysmal hypertensive attacks. Because of high metabolic pressure on circulating catecholamines, measurements of urinary metabolites in either 24-h urines or spontaneous voidings (notably after paroxysmal attacks) may offer both highest specificity and sensitivity to establish increased catecholamine production rate. Unlike urinary total catecholamines, urinary total metanephrine levels are not significantly influenced by diet [17]. Deactivation of circulating catecholamines by initial 3-O-methylation followed by sulphoconjugation and excretion suggests highest sensitivity and specificity for urinary total metanephrines, compared with oxidatively deaminated catecholamine metabolites. Therefore, determination of urinary total metanephrines is generally considered to be the principal test for the clinical chemical diagnosis of phaeochromocytoma [3,18].

Several analytical techniques have been developed that either quantify the sum of urinary total metanephrines or selectively determine the 3-O-methylated catecholamines 3-MT, NM and M. These include: electrophoresis [19], paper [20] and thin-layer [21] chromatography with subsequent chromogen formation and spectrophotometric detection, ion-exchange chromatography followed by vanillin formation and spectrophotometric detection [22], fluorescent derivatization [23], radioenzymatic quantification of NM [24], reversed-phase high-performance liquid chromatography with fluorimetric [25,26], ultraviolet-absorbance [27] or electrochemical [28,29] detection, and gas chromatography with mass spectrometric detection [16,30].

Adhering to the principle of isotope dilution for urinary total 3-O-methylated catecholamine metabolites, we developed an alternative method to our previously described prepurification method by acid hydrolysis and alkaline extraction, followed by electron-impact mass fragmentography of their pentafluoropropionyl (PFP) derivatives.

EXPERIMENTAL

Standards and reagents

HPLC-grade acetonitrile and heptane were obtained from Rathburn (Walkerburn, UK), pen-

tafluoropropionic anhydride (PFPA) and dimethylformamide (DMF, silylation grade) from Pierce (Rockford, IL, USA), ethyl acetate was from Merck (Darmstadt, Germany), and 3-MT, NM and M were from Sigma (St. Louis, MO, USA). All reagents were at least of analytical-reagent grade.

Samples

For assessment of excretion values of 3-MT, NM and M, urine samples from nineteen apparently healthy adults (ages 27–63 years) were collected without dietary restrictions and in an undefined metabolic state. Total urine outputs over 24 h were collected in 2-l brown polypropylene bottles (Sarstedt, Nuembrecht, Germany), containing about 250 mg each of $Na_2S_2O_5$ and EDTA as preservatives. Urine specimen were acidified to pH 4.0 with acetic acid before freezing. Samples were stored at −20°C and analysed within one month after collection.

Urinary creatinine levels, used to quantify excretion in terms of creatinine, were measured by a picric acid method on an SMA-2 analyser (Technicon Instruments, Tarrytown, FL, USA).

Prepurification

Deuterated analogues of 3-MT (550 pmol of ring-deuterated, prepared as described by Lindström *et al.* [31]) and NM and M (900 and 600 pmol, respectively; C^2H_3O-deuterated, prepared as previously described [32]) were added to 300-μl aliquots of urine as internal standards. After lyophilization overnight (or evaporation to dryness in a stream of nitrogen at 40°C), 100 μl each of acetonitrile (distilled and stored over anhydrous sodium sulphate), DMF and PFPA were added. The mixtures were derivatized for 15 min at 80°C in sealed vials. Samples were cooled to room temperature and aliquots of 2 ml of heptane and 2 ml of water were added. Following vortexing for 30 s and centrifugation at 800 *g* for 5 min, the heptane layers were collected, washed with 2 ml of water as above, and evaporated to dryness in a stream of nitrogen at 40°C. Residues were dissolved in 50 μl of ethyl acetate–PFPA (250:1, v/v). Volumes of 1–5 μl were injected into the gas chromatograph–mass spectrometer (see below).

Analysis and quantification

Gas chromatography–mass fragmentography was performed on an HP-5890 gas chromatograph (Hewlett Packard, Avondale, PA, USA) directly coupled to a VG-70 250-S mass spectometer (VG Instruments, Manchester, UK). The gas chromatograph was equipped with a 25 m × 0.25 mm I.D. CP-Sil 19 capillary column (Chrompack, Middelburg, Netherlands), and operated under the following conditions: splitless injection, injection temperature 250°C, oven temperature programme 100°C, 10°C/min to 230°C. The ion source temperature of the mass spectrometer was 250°C; ionization energy was 120 eV. Samples were monitored in the ammonia chemical ionization mode, using the $[M + NH_4]^+$ ions at *m/z* 477 and 480 (3-MT and 3-MT-d_3, respectively), *m/z* 639 and 642 (NM and NM-d_3, respectively) and *m/z* 653 and 656 (M and M-d_3, respectively). The peak area ratios of 477/480, 639/642 and 653/656 at the retention times corresponding to PFP-derivatized 3-MT, NM and M, respectively, were calculated by a VG 11-250 data system.

Quantification was done by the use of calibration curves, as previously described [16]. Calibration curves were prepared from the peak area ratios of seven derivatized standards containing 0–720 pmol of 3-MT, 0–1300 pmol of NM and 0–915 pmol of M, added to 550 pmol of 3-MT-d_3, 900 pmol of NM-d_3 and 600 pmol of M-d_3.

Validation of the assay

The detection limit was determined by subsequent analysis of a urine sample that was progressively diluted with water. The detection limit was defined as the injected amount that produced a signal-to-noise ratio of 3. Linearity was verified by analyses of standard mixtures containing increasing amounts of 3-MT, NM and M added to 550 pmol of 3-MT-d_3, 900 pmol of NM-d_3 and 600 ng of M-d_3.

Within-series precision was determined by six-fold analysis of three urine samples of different concentrations in one series. Within-series recovery was determined by six-fold analysis of a 300-μl urine sample with or without enrichment

with 240 pmol of 3-MT, 440 pmol of NM and 305 pmol of M in one series. Between-series precision was evaluated by eight-fold analysis of four urine samples of different concentrations, distributed over eight series, in a period of one month. Between-series recovery was assessed by eight-fold analysis of a 300-μl urine sample with or without enrichment with 240 pmol of 3-MT,

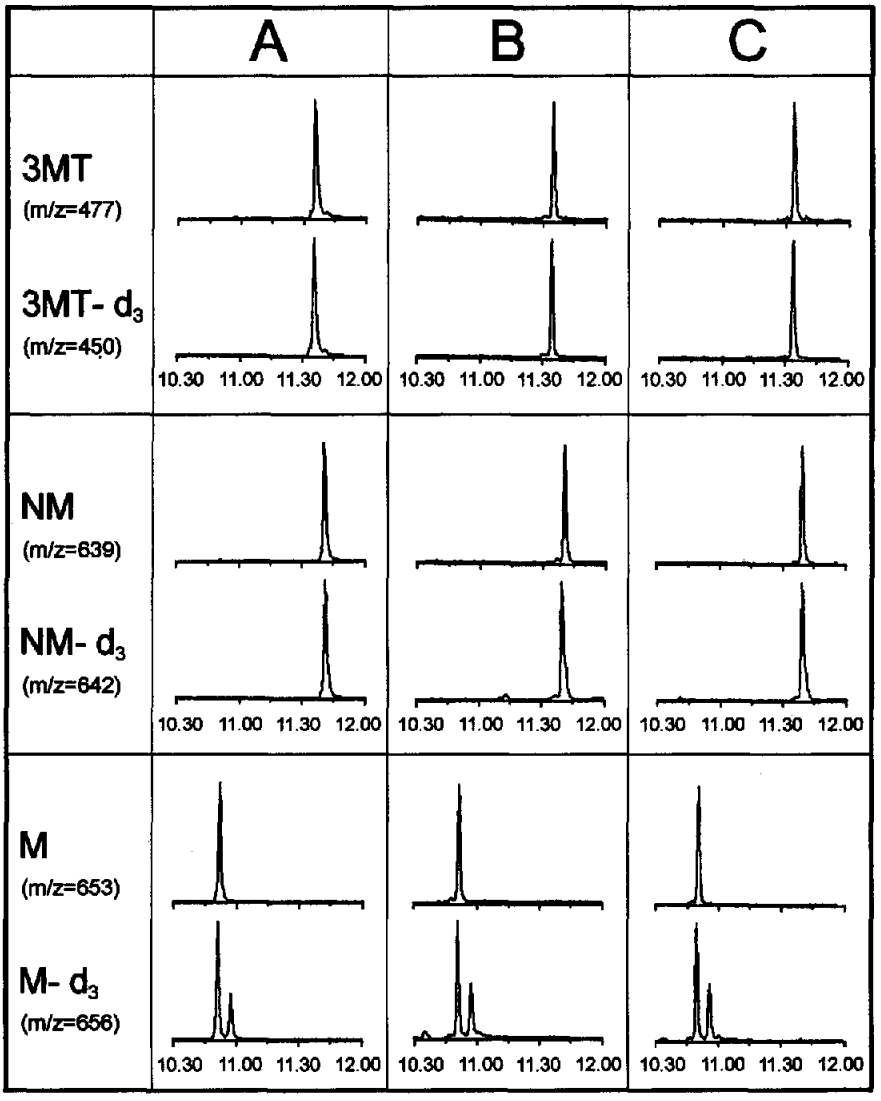

Fig. 1. Typical ammonia chemical ionization mass fragmentograms of PFP-derivatized 3-methoxytyramine (3-MT), normetanephrine (NM) and metanephrine (M), and their trideuterated internal standards in a 600-μl standard mixture (A) and 300-μl aliquots of urine from an apparently healthy adult (B) and a patient with phaeochromocytoma (C). The signals are normalized to full scale for the highest peak in the window. Retention time is indicated in min. 3-MT, NM, M, 3-MT-d_3, NM-d_3 and M-d_3 denote $[M + NH_4]^+$ ions of the naturally occurring compounds and their trideuterated internal standards, respectively. In the mass fragmentograms at *m/z* 656, the minor peak at higher retention time represents iso-M-d_3, which is formed during synthesis of M-d_3. Iso-NM-d_3 and NM-d_3 co-elute. Since 3-MT-d_3 is synthesized from 3-MT by ring deuteration, 3-MT-d_3 contains no impurity of iso-3-MT-d_3. Concentrations of the standard mixture and the urine samples were (A) 3-MT 600, NM 1100 and M 760 nmol/l; (B) 3-MT 65, NM 85 and M 37 μmol/mol of creatinine; (C) 3-MT 98, NM 662 and M 619 μmol/mol of creatinine. In all three cases 550, 900 and 600 pmol of the trideuterated forms of 3-MT, NM and M, respectively, were added as internal standards.

440 pmol of NM and 305 pmol of M, in eight series equally distributed over a period of two months.

Statistics

Confidence intervals (95%) were calculated by non-parametric analysis [33].

RESULTS

Chromatography

Fig. 1 shows typical mass fragmentograms of PFP-derivatized 3-MT, NM and M, and their trideuterated internal standards in a standard mixture (Fig. 1A), a 300-μl aliquot of urine from an apparently healthy adult (Fig. 1B) and a patient with phaeochromocytoma (Fig. 1C).

Methodological characteristics

The amounts of urinary constituents in a 300-μl aliquot of lyophilized urine harbouring up to about 4.5 μmol of creatinine (corresponding to a urinary creatinine concentration of about 15 mmol/l) had no adverse effects on the derivatization reaction. However, if the amounts exceeded 6 μmol of creatinine equivalents, recoveries were found to become progressively lower (as derived from mass fragmentographic peak areas), whereas the contribution of compounds with the same fragment ions eluting at different retention time became higher. In these (rare) cases, a proportionally lower sample volume was taken. Compared with overnight lyophilization, similar results were obtained by addition of 300 μl of methanol and evaporation to dryness under a stream of nitrogen at 40°C. Derivatization time was varied from 5 up to 30 min. From both mass fragmentographic peak areas and their ratios it was concluded that the reaction was completed in 10 min. As a standard procedure we chose 15 min. Overnight storage of the washed heptane layer did not show any appreciable influence on the analytical outcome, compared with direct analysis. Rederivatization of phenolic and benzylic hydroxyl groups increased mass fragmentographic peak areas, but did not alter peak area ratios.

Validation of the assay

Detection limits (signal-to-noise ratio = 3; data in pmol of injected compound from diluted urine) for 3-MT, NM and M were 0.3, 2.6 and 0.3 pmol on-column, respectively. For 3-MT and M these amounts corresponded to a urine volume of about 10 μl; for NM the detection limit was reached at about 50 μl of urine. The endogenous concentrations of the urine sample were 980, 1602 and 881 nmol/l for 3-MT, NM and M, respectively. Calibration curves, prepared from eight derivatized standards containing 0–1075 pmol of 3-MT, 0–1970 pmol of NM and 0–1370 pmol of M and fixed amounts of 550 pmol of 3-MT-d_3, 900 pmol of NM-d_3 and 600 pmol of M-d_3 gave linear correlations ($r > 0.999$).

Table I shows the within-series precision for three urine samples with different concentrations of 3-O-methylated catecholamine metabolites. The coefficients of variation ranged from 1.7 to 4.2%. Variance in creatinine results was not accounted for. Within-series recoveries were deter-

TABLE I

PRECISION AND REPRODUCIBILITY OF URINARY 3-METHOXYTYRAMINE (3-MT), NORMETANEPHRINE (NM) AND METANEPHRINE (M)

Sample	Concentration (μmol/mol of creatinine)		
	3-MT	NM	M
Within-series precision[a]			
NP1	80 ± 2	115 ± 3	84 ± 2
NP2	101 ± 2	143 ± 6	85 ± 2
P1	90 ± 2	576 ± 10	682 ± 14
Between-series precision[b]			
NP3	58 ± 3	152 ± 11	97 ± 6
NP4	92 ± 7	268 ± 12	103 ± 5
P2	91 ± 14	597 ± 37	592 ± 49
P3	102 ± 8	581 ± 19	577 ± 39

[a] Mean ± S.D. for three urine samples from non-phaeochromocytoma patients (NP1, NP2) and a patient with phaeochromocytoma (P1) determined six-fold in one series.

[b] Mean ± S.D. for four urine samples from two non-phaeochromocytoma patients (NP3, NP4) and two patients with phaeochromocytoma (P2, P3) each determined eight-fold in eight series equally distributed over a period of one month.

TABLE II

URINARY EXCRETION VALUES FOR 3-METHOXYTYRAMINE (3-MT), NORMETANEPHRINE (NM) AND METANEPHRINE (M) ESTABLISHED FOR NINETEEN APPARENTLY HEALTHY ADULTS

Data are indicated as median (95% non-parametric confidence interval).

Compound	Extractive derivatization[a]		Alkaline extraction[b] (μmol/mol creatinine)
	μmol/mol creatinine	nmol/24 h	
3-MT	86 (58–139)	1183 (878–2056)	66 (40–141)
NM	141 (82–234)	2053 (1561–3379)	112 (71–248)
M	66 (38–87)	911 (574–1296)	40 (26–65)

[a] Data established in nineteen apparently healthy adults by isotope-dilution mass fragmentographic analysis preceded by direct derivatization and extraction.

[b] Data (recalculated from Muskiet *et al.* [16]) established in thirteen apparently healthy adults by isotope-dilution electron impact mass fragmentographic analysis preceded by acid hydrolysis, alkaline extraction and derivatization.

mined by six-fold analysis of a urine sample with and without enrichment with 800 nmol/l 3-MT, 1466 nmol/l NM and 1017 nmol/l M. The recoveries were (mean ± S.D.) 88.5 ± 5.6% for 3-MT, 85.1 ± 3.1% for NM and 100.5 ± 10.7% for M. The endogenous levels in the urine sample (mean ± S.D., in nmol/l) were 1213 ± 25 (3-MT), 1712 ± 77 (NM) and 1025 ± 29 (M).

Table I shows the between-series precision for four urine samples with different concentrations of 3-O-methylated catecholamine metabolites. The coefficients of variation ranged from 3.3 to 15.4%. Between-series recoveries were determined by eight-fold analysis of a single urine sample, with and without enrichment. The analyses were performed in eight series, equally distributed over a period of two months. Enrichment amounted to 800 nmol/l 3-MT, 1466 nmol/l NM and 1017 nmol/l M. The recoveries were (mean ± S.D.): 100.2 ± 14.5% for 3-MT, 91.2 ± 12.3% for NM and 97.7 ± 6.8% for M. The endogenous levels in the urine sample (mean ± S.D., in nmol/l) were 497 ± 48 (3-MT), 2567 ± 260 (NM) and 719 ± 63 (M).

Urinary excretion values

Table II shows median urinary excretion values for 3-MT, NM and M, together with non-parametric 95% confidence intervals for nineteen healthy adults (median age 41 years, range 27–63), as obtained by the direct derivatization method. Data are given in μmol/mol of creatinine and nmol/24 h. The results are compared with urinary excretion values (in μmol/mol of creatinine) that were obtained by the method employing acid hydrolysis and alkaline extraction. [16].

DISCUSSION

Several procedures have been described for the isolation of metanephrines from biological matrices. These include ion-exchange chromatography [25,34], (paired ion) solvent extraction [16,35], solid-phase extraction [30] and extractive derivatization [36,37]. For chromatographic methods that require initial derivatization and injection from an organic solvent, such as gas chromatography, the usual procedure is to isolate metanephrines in a water- and salt-free form prior to derivatization. This is necessary because of the susceptibility of commonly employed derivatization reagents to water and inhibition of the reaction by salts. Because of the somewhat amphoteric nature of metanephrines only a few prepurification methods seem appropriate to reach this goal, *i.e.* organic solvent [16] or solid phase extraction [30], both at high pH, and extractive derivatization in which the amine functional group is blocked and the amide or amide-like products extracted into an organic solvent

[36,37]. Our previously described method, which we used routinely for over 10 years, was based on ethyl acetate extraction of urine at pH 10–12. The main disadvantage of this approach is its low absolute recovery (notably for NM), probably caused by partial oxidation of metanephrines at high pH and saponification of ethyl acetate. The latter unfavourably influences organic solvent/aqueous medium ratio and reduces pH. Although not of crucial importance to a method based on isotope dilution, low absolute recovery, especially for NM, occasionally resulted in peak areas that were close to the detection limit. Improvement of recovery by use of solid-phase C_{18} columns [30] necessitated adjustment of samples to pH 8–9 to obtain reasonable recoveries, especially for NM. This pH is close to the point of C_{18} column deterioration. Extractive derivatization methods based on the addition of methyl chloroformate [37] and PFPA [36] have been reported. The reaction with methyl chloroformate occurs at two different pH values and seems rather critical and laborious, whereas the reaction with PFPA requires a temperature of 0°C to prevent excessive PFPA decomposition in aqueous medium.

In the present method addition of the water-sensitive derivatization reagent to the aqueous medium is circumvented by initial removal of water through lyophilization or evaporation to dryness. Up to a urinary creatinine concentration of 15 mmol/l no inhibitory effects of salts and other urinary constituents on derivatization with a mixture of PFPA–acetonitrile–DMF were experienced. Weak combinations of a strong acid and slightly acidic hydroxyl groups, as constituted by PFP-derivatized phenolic or benzylic hydroxyl groups, may hydrolyse during the subsequent extraction in which urinary constituents are partitioned between water and heptane. A second derivatization at room temperature, comprising addition of ethyl acetate–PFPA prior to analysis, was found to replenish PFP groups.

Since it is known that the major part (56–87%, ref. 35) of urinary metanephrines is conjugated, deconjugation prior to analysis is a standard procedure. This is usually accomplished either by acid hydrolysis at high temperatures [22] or by enzymatic hydrolysis with sulphatase [35]. Using enzymatic hydrolysis with sulphatase, Buu *et al.* [35] compared their data on the sum of free and sulphoconjugated urinary NM and M with those obtained by other laboratories that employed acid hydrolysis. From the close agreement they concluded that the vast majority of O-methylated catecholamine conjugates in human urine are sulphoconjugated. Alton and McGoodall [38] estimated the contribution of NM glucuronide to be approximately 4%, whereas the percentage M glucuronide was probably somewhat higher [39]. During preliminary experiments we found that results of the direct derivatization procedure were in close agreement with those of the acid hydrolysis–alkaline extraction procedure. We therefore anticipated that the direct derivatization procedure was able to transesterify metanephrine sulphate esters into their PFP esters. To confirm this we performed an experiment in which results of the direct derivatization method were systematically compared with those obtained from preceding acid hydrolysis for 30 min at pH 1 and 90°C, followed by the standard direct derivatization method. From the obtained close similarity of results from ten urine samples it was concluded that the direct derivatization method is indeed capable of deconjugating metanephrine sulphates. It is unlikely that the more stable ether bonds in metanephrine glucuronides are hydrolysed quantitatively. In this respect solvolysis does not seem to differ from acid hydrolysis, since the latter also causes incomplete glucuronide deconjugation [39]. In addition we found that, compared with preceding acid hydrolysis, mass fragmentograms from directly derivatized urine samples showed higher absolute peak areas and fewer compounds giving rise to the same fragment ions at different retention times. These data suggest higher absolute recovery and less interference, respectively, in the direct derivatization method. Omission of the hydrolysis and alkaline extraction steps in the direct derivatization procedure significantly reduces the required time for prepurification.

Table II shows that median urinary excretion

values obtained with the present method are somewhat higher than those previously reported, using the acid hydrolysis–alkaline extraction method [16]. Although the reason for the discrepancy is, as yet, not fully established, the following observations offer some connecting points. Experiments in which urines were enriched with NM and M prior to acid hydrolysis showed that this procedure causes low recovery of added standards, compared with the direct derivatization method (data not shown). As previously established by others [39–41], experimental conditions for acid hydrolysis of urinary metanephrines are critical. Factors such as pH, temperature and hydrolysis time influence the outcome substantially. Decomposition of NM and M takes place at pH values below 1, and when hydrolysis time exceeds 30 min [41]. It suggests that methods based on acid hydrolysis and subsequent addition of internal standards are subject to incomplete recovery of total metanephrines, resulting in somewhat lower excretion values. Low recovery from acid hydrolysis does not explain the higher median excretion values for 3-MT by the direct derivatization procedure, since 3-MT is a highly acid-stable compound. In this case the most likely explanation is additional measurement of 4-O-methyldopamine (iso-3-MT) with the present method. Analogous to iso-HVA [2], contribution of iso-3-MT may be of the order of 6%. As previously reported [32], iso-3-MT is poorly extracted from alkalinized medium, resulting in low recovery in our previously described method. By virtue of iso-3-MT isolation in the direct derivatization method and similar gas chromatographic retention times of PFP-derivatized 3-MT and iso-3-MT, higher values for 3-MT with the direct derivatization method may be anticipated. The magnitude of the discrepancy will depend on both the relative contribution of iso-3-MT to 3-MT in the sample, and the difference in the yields of the m/z 477 ions from equimolar amounts of PFP-derivatized iso-3-MT and 3-MT. Also when compared with other reference data established with mass fragmentographic analysis [30], our reference group showed higher values for NM and M. However, reference data established by HPLC with electrochemical detection [42] show comparable data for NM and M.

The employed prepurification method is specific for those compounds that display apolar properties following direct PFP derivatization and subsequent contact with water, *i.e.* notably amines and possibly sugars. Except for the isolation of 3-MT, NM and M this approach was also found (data not shown) to recover DA, NE and E, and the products of the alternative catecholamine biosynthetic pathway, synephrine, octopamine, epinine and tyramine (false neurotransmitters). However, as might have been expected for compounds harbouring tertiary amine groups, NME and MM were poorly recovered. In the present application the required specificity is introduced by the use of mass fragmentographic detection. Monitoring of other fragment ions, together with those of appropriate internal standards, offers the possibility to use the same isolation method for analyses of a wide variety of biologically occurring mono-, di- and polyamines. Alternatively, by employing gas chromatography with electron-capture or nitrogen–phosphorus detection, the isolation procedure may enable development of a more generally applicable profiling method.

In conclusion, we developed a prepurification procedure for the quantification of urinary 3-O-methylated catecholamines by isotope-dilution chemical ionization mass fragmentography. The prepurification method may prove applicable in the development of profiling methods for a wide variety of biologically occurring amines.

REFERENCES

1 I. J. Kopin, *Pharmacol. Rev.*, 37 (1985) 333.
2 F. A. J. Muskiet, D. C. Fremouw-Ottevangers, G. T. Nagel and B. G. Wolthers, *Clin. Chem.*, 25 (1979) 1708.
3 T. G. Rosano, T. A. Swift and L. W. Hayes, *Clin. Chem.*, 37 (1991) 1854.
4 L. Landsberg and J. B. Young, in J. D. Wilson and D. W. Foster (Editors), *Williams Textbook of Endocrinology*, Saunders, Philadelphia, PA, 1985, Ch. 23, p. 891.
5 R. J. Wurtman and J. Axelrod, *J. Biol. Chem.*, 241 (1966) 2301.
6 R. R. Robinson, *Tumours that Secrete Catecholamines: their Detection and Clinical Chemistry*, Wiley, Chichester,1980.

7 J. A. Christofides and D. E. Frey, *Ann. Clin. Biochem.*, 25 (1988) 119.
8 P. A. Tippet, A. J. McEwan and D. Mackery, *Clin. Endocrinol.*, 25 (1986) 401.
9 C. Itoh, K. Yoshinaga, T. Sato, N. Ishida and Y. Wada, *Nature*, 193 (1962) 477.
10 R. Robinson, P. Smith and S. R. F. Whittaker, *Br. Med. J.*, 1 (1964) 1422.
11 J. Gerlo and R. Malfait, *J. Chromatogr.*, 414 (1987) 301.
12 A. M. Puyo, G. M. Levin, I. Armando and M. B. Barontini, *Acta Endocrinol. (Copenhagen)*, 113 (1986) 111.
13 E. L. Bravo, R. C. Tarazi, R. W. Gifford and B. H. Stewart, *N. Engl. J. Med.*, 301 (1979) 682.
14 M. W. Duncan, P. Compton, L. Lazarus and G. A. Smythe, *N. Engl. J. Med.*, 319 (1988) 136.
15 D. H. Jones, J. L. Reid, C. A. Hamilton, D. J. Allison, R. B. Welbourn and C. T. Dollery, *Q. J. Med.*, 195 (1980) 341.
16 F. A. J. Muskiet, C. G. Thomasson, A. M. Gerding, D. C. Fremouw-Ottevangers, G. T. Nagel and B. G. Wolthers, *Clin. Chem.*, 25 (1979) 453.
17 P. Moleman, *Clin. Chim. Acta*, 189 (1990) 19.
18 M. Mannelli, *J. Endocrinol. Invest.*, 12 (1989) 739.
19 K. Yoshinaga, C. Itoh, N. Ishida, T. Sato and Y. Wada, *Nature*, 191 (1961) 599.
20 R. F. Coward and P. Smith, *Clin. Chim. Acta*, 14 (1966) 672.
21 J. M. C. Gutteridge, *Clin. Chim. Acta*, 21 (1968) 211.
22 J. J. Pisano, *Clin. Chim. Acta*, 5 (1960) 406.
23 B. L. Biglow and H. Weil-Malherbe, *Anal. Biochem.*, 26 (1968) 92.
24 N. D. Vlachakis and A. Niarchos, *Clin. Chim. Acta*, 99 (1979) 283.
25 N. G. Abeling, A. H. van Gennip, H. Overmars and P. A. Vôute, *Clin. Chim. Acta*, 137 (1984) 211.
26 G. P. Jackman, *Clin. Chim. Acta*, 120 (1982) 137.
27 J. Bieva, I. H. Ladmirant, I. Scheirs and J. P. M. Dardenne, *Clin. Chem.*, 33 (1987) 1677.
28 R. E. Shoup and P. T. Kissinger, *Clin. Chem.*, 23 (1977) 1268.
29 R. Pagliari, J. M. Cottet-Emard and L. Peyrin, *J. Chromatogr.*, 563 (1991) 23.
30 P. C. Canfell, S. R.Binder and H. Kayam-Bashi, *Clin. Chem.*, 28 (1982) 25.
31 B. Lindström, B. Sjöquist and E. Änggärd, *J. Labelled Compd.*, 10 (1974) 187.
32 F. A. J. Muskiet, S. Pavel, G. J. Stob and H. Elzinga, *J. Labelled Compd.*, 20 (1983) 627.
33 H. E. Solberg, *Clin. Chim. Acta*, 170 (1987) S13.
34 P. Volin, *J. Chromatogr.*, 578 (1992) 165.
35 N. T. Buu, M. Angers, D. Chevalier and O. Kuchel, *J. Lab. Clin. Med.*, 104 (1984) 425.
36 O. Beck and K. F. Faull, *Anal. Biochem.*, 149 (1985) 492.
37 A. P. J. M. de Jong, *J. Chromatogr.*, 276 (1983) 267.
38 H. Alton and C. McGoodall, *Biochem. Pharmacol.*, 17 (1968) 2163.
39 E. R. B. Smith and H. Weil-Malherbe, *J. Lab. Clin. Med.*, 60 (1962) 212.
40 H. Weil-Malherbe, in D. Glick (Editor), *Analysis of Biogenic Amines and their Related Enzymes (Methods of Biochemical Analysis*, suppl. vol.), Wiley-Interscience, New York, 1971, pp. 119–152.
41 P. Moleman and J. J. M. Borstrok, *Biogenic Amines*, 3 (1985) 33.
42 V. Kairisto, P. Koshinen, K. Matilla, J. Puikkonen, A. Virtanen and K. Irjala, *Clin. Chem.*, 38 (1992) 416.