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Evaluation of degradation of urinary catecholamines and metanephrines and deconjugation of their sulfoconjugates using stability-indicating reversed-phase ion-pair HPLC with electrochemical detection

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Abstract

The possibility of the deconjugation of urinary catecholamine sulfates (CA-S) and metanephrine sulfates (MN-S) during storage was studied using a reversed-phase ion-pair HPLC method with electrochemical detection. Stability profiles of catecholamines (norepinephrine (NE), epinephrine (E) and dopamine (DA)) and metanephrines (normetanephrine (NM) and metanephrine (MN)) in preserved and unpreserved pooled urine and aqueous samples stored at 10 and 30°C over 8 days were compared. Results showed that these compounds exhibited stable or declining profiles in aqueous samples but fluctuating profiles in pooled urine samples. It was concluded that deconjugation of CA-S and MN-S occurred in both preserved and unpreserved urine samples. Therefore, at 10 and 30°C, levels of detected urinary catecholamines and metanephrines could be affected by both the degradation of the free amines and deconjugation of the CA-S and MN-S. Based on these findings, another batch of unpreserved urine and aqueous samples were prepared and stored at − 80°C. Under this condition, all compounds were found to be stable with less than 10% variations in both unpreserved urine and aqueous samples for at least 22 days. These indicated that urine samples could be stored unpreserved at − 80°C for at least 3 weeks without significant degradation of the free amines or deconjugation of their sulfoconjugates. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Catecholamine sulfates; Metanephrine sulfates; Electrochemical detection; Deconjugation; Degradation

1. Introduction

Accurate and specific measurements of catecholamines, metanephrines, CA-S and MN-S in biological specimens are important in both the clinical diagnosis and pathological studies of certain diseases. Pheochromocytoma is a tumor of chromaffin cells that secrete catecholamines. These catecholamines are converted by catechol-*o*-methyltransferase (COMT) into metanephrines and 3-methoxytyramine (Fig. 1). The clinical condition of pheochromocytoma is usually presented

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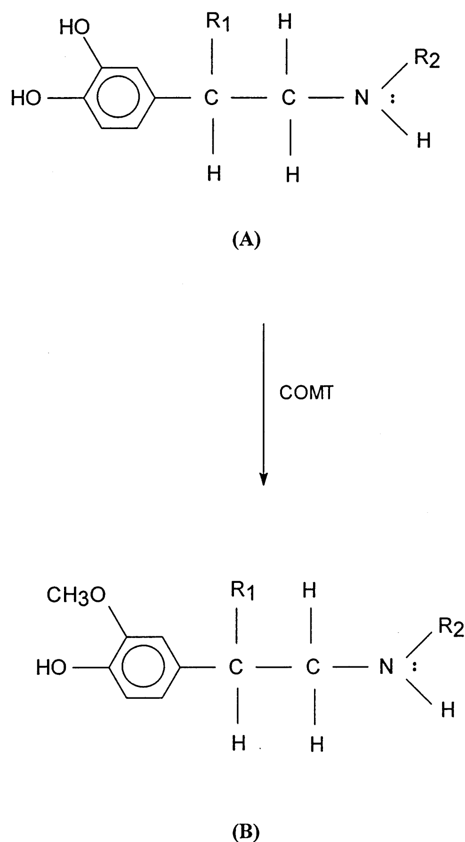
as hypertension. It has been estimated that up to 50% of these cases were not diagnosed in life [1]. The early diagnosis of pheochromocytoma is important, not only because it offers the possibility of curing the condition but also because unrecognized pheochromocytoma is a potentially lethal condition. Estimation of urinary free catecholamines and total metanephrines is routinely used in the biochemical detection of pheochromocytoma and in monitoring the completeness of tumor excision as well as the possibility of recurrence [2]. Measurement of urinary dopamine (DA), along with norepinephrine (NE) and epinephrine (E), is especially useful in the diagnosis of malignant forms of the tumor [2].

CA-S which occur in higher concentrations than free catecholamines in human plasma, urine [3,4] and cerebrospinal fluid [5] are suggested as the transport form of the catecholamines [6]. Therefore, CA-S are also of topical interest with regard to clinical disorders such as parkinsonism [7], hypertension [8] and pheochromocytoma [9,10]. Puyo et al. [9] commented that sulfoconjugation of catecholamines plays an important role



	Compounds	R_1	R_2
(A)	NE	OH	H
	E	OH	CH_3
	DA	H	H
(B)	NM	OH	H
	MN	OH	CH_3
	3-MT	H	H

Fig. 1. Metabolic path of catecholamines.

in determining the hypertension pattern in pheochromocytoma patients. Vandongen et al. [10] found that sulfate conjugation is a dynamic process that may limit wide fluctuations in free catecholamine concentration during episodic secretory activity in pheochromocytoma.

MN-S also occur in higher concentrations in human urine than their free amines. Buu et al. [1] showed that differentiation between free and sulfoconjugated metanephrines might be useful in the study of the activity of the sympathetic nervous system and in the clinical evaluation of the quantities of excreted metabolites. Because of the importance of accurate measurements of free catecholamines, metanephrines and their sulfoconjugates, any process that causes deconjugation of CA-S and MN-S in biological samples should therefore be determined and suitably circumvented.

Catecholamines and metanephrines are prone to oxidation [12]. Various authors have reported stability studies of catecholamines in biological fluids [13–17]. Boosman et al. [16] observed and attributed the chaotic stability profile of these analytes in plasma and urine to the depletion of antioxidant added to the biological samples. Miki and Sudo [14] reported in their study that unpreserved urine samples could be used for the measurement of catecholamines, cortisol and creatinine. However, they failed to comment on the increases in measured concentrations of catecholamines (greater than 10%) in acidified urine samples (pH 1 and 0.5) during storage at room temperature (15–20°C). Metanephrines were not measured in these studies [13–16] and the methods used were also not validated to be stability-indicating. Furthermore, none of these authors explored the possibility of deconjugation of CA-S and MN-S in unpreserved and preserved urine samples.

The objective of this study was to use a reversed-phase ion-pair HPLC method with electrochemical detection to determine the potential of deconjugation of CA-S and MN-S and the stability of the unconjugated compounds in unpreserved and preserved pooled urine samples during storage. This would give us an insight on the contribution of deconjugation if occurred to the measured concentrations of NE, E, DA, normetanephrine (NM) and metanephrine (MN) in urinary samples.

2. Experimental

2.1. Materials

All reagents were of analytical grade and Milli-Q reagent water was used. NE, E, DA, NM, MN, 3,4-dihydroxybenzylamine (DHBA, internal standard), Na_2EDTA, sodium metabisulfite were obtained from Sigma (St. Louis, MO, USA). Sodium 1-heptanesulfonate (ion-pair), Bio-Rex 70 cation-exchange resin and poly-prep columns for solid phase extraction were purchased from Bio-Rad Labs (Hercules, CA, USA).

2.2. Instrumentation

The liquid chromatographic system, model LC-10AT (Shimadzu Co., Kyoto, Japan), included a solvent delivery system, a low-pressure gradient flow control valve and an on-line degasser. The electrochemical detector, model L-ECD-6A (Shimadzu), consisted of a glassy carbon working electrode and a silver/silver chloride reference electrode. Two serially connected Nova-Pak C_{18} columns (each 3.9×150 mm, 4 μm particle size) were purchased from Waters (Milford, MA, USA). A perspex cabinet and a water jacket were used to maintain constant operating temperatures of 32 and 52°C for the detector and the columns, respectively. The injector was fitted with a 100 μl loop.

2.3. HPLC conditions

A running buffer solution, composed of 200 mmol l^{-1} $NaH_2PO_4 \cdot H_2O$, 4 mmol l^{-1} sodium 1-heptanesulfonate and 0.2 g l^{-1} Na_2EDTA was adjusted to pH 3.0 with 1 mol l^{-1} orthophosphoric acid, then filtered through a 0.45 μm filter (Millipore, Bedford, MA, USA). Acetonitrile (2.2%) was mixed on-line with 97.8% of the running buffer. The flow rate was 1.2 ml min^{-1}. A potential of $+0.85$ V was applied across the electrodes of the detector.

2.4. Urine and aqueous samples

Urine samples were collected from healthy subjects and pooled in an amber glass bottle stored at −20°C. All the subjects were abstained from medications for 3 days prior to urine collection. The pooled urine sample (1 l) was allocated to each of the three amber glass bottles containing either no additive; 5 ml of 3 M HCL or 0.5 g each of Na_2EDTA and sodium metabisulfite as preservatives. These urine samples were carefully mixed and aliquots from each bottle were transferred to 24 polyethylene tubes. Twelve of them were kept at 10°C and the other 12 stored at 30°C. Three tubes from these two batches of samples were taken out on days 1, 3, 5 and 8 for the analysis of catecholamines and metanephrines. Triplicate samples from each group were also extracted and analyzed on day 0. Similarly, aqueous samples containing 200 nmol l^{-1} of NE, E, DA, NM and MN were subjected to the three preserving conditions under the two storage temperatures described as for the urine specimens. Samples were analyzed on days 0, 1, 3, 5 and 8 to monitor the stability of catecholamines and metanephrines in aqueous solution.

Based on the findings of this study, batches of unpreserved pooled urine and aqueous samples were prepared and stored at −80°C. Duplicate urine and aqueous samples were analyzed on days 1, 3, 5, 8, 15, 22 and 29. Triplicate samples from the pooled urine and aqueous samples were analyzed on day 0.

2.5. Sample preparation

Simultaneous extraction of NE, E, DA, NM and MN in urine and aqueous samples using Bio-Rex 70 cation exchange resin was based on the method of Chan and Siu [18]. A 5 ml aliquot of urine sample, aqueous sample or calibrator was transferred to a centrifuge tube and mixed with 5 μl of internal standard (400 μmol l^{-1} of DHBA in 0.1 M HCL), 100 μl of Na_2EDTA solution (0.1 g ml^{-1}) and 100 μl of freshly prepared sodium metabisulfite (0.1 g ml^{-1}). The pH of the mixture was adjusted to 6.5 ± 0.1 by adding 1 M NaOH. The mixture was transferred to the pre-conditioned Bio-Rex 70 resin packed in the poly-prep column and allowed to drain. The column was washed with 10 ml of Milli-Q water and eluted with 7 ml of 4 M formic acid. The eluate was collected and a 250-μl aliquot was injected directly into the column.

2.6. Extent of sulfoconjugation in spot urine samples

Four spot urine samples were also collected in the morning before breakfast from four subjects. One of these subjects has essential hypertension (male, aged 59) and one exercised for 1 h before urine collection (male, aged 27). The other two subjects are healthy (female, aged 27 and 50). The CA-S and MN-S in these samples were deconjugated as described in a previous study [19]. Briefly, a 5-ml aliquot of each spot urine sample was mixed with 100 μl of Na_2EDTA solution (0.1 g ml^{-1}) and 100 μl of sodium ascorbate solution (0.05 g ml^{-1}). The sample was adjusted to pH 1.0 ± 0.1 with 3 M HCL, and then heated at 100°C for 7 min. The sample was cooled and mixed with 100 μl of Na_2EDTA solution (0.1 g ml^{-1}), 100 μl of freshly prepared sodium metabisulfite (0.1 g ml^{-1}) and 5 μl of DHBA solution (400 μmol l^{-1}); and was extracted and analyzed as described. To determine the amount of free urinary amines, a 5 ml aliquot of the spot urine sample was analyzed without acid hydrolysis. To determine the amount of catecholamines and metanephrines lost during hydrolysis, duplicates of aqueous samples were analyzed with and without acid hydrolysis.

3. Results and discussion

3.1. Method validation

Using the developed chromatographic conditions, peaks of NE, E, DA, NM, MN and DHBA were resolved in urine sample (Fig. 2). No significant endogenous peak was found at the retention time of the internal standard (DHBA). Acid metabolites, alcoholic metabolites and amino acids were not retained by the cation-exchange

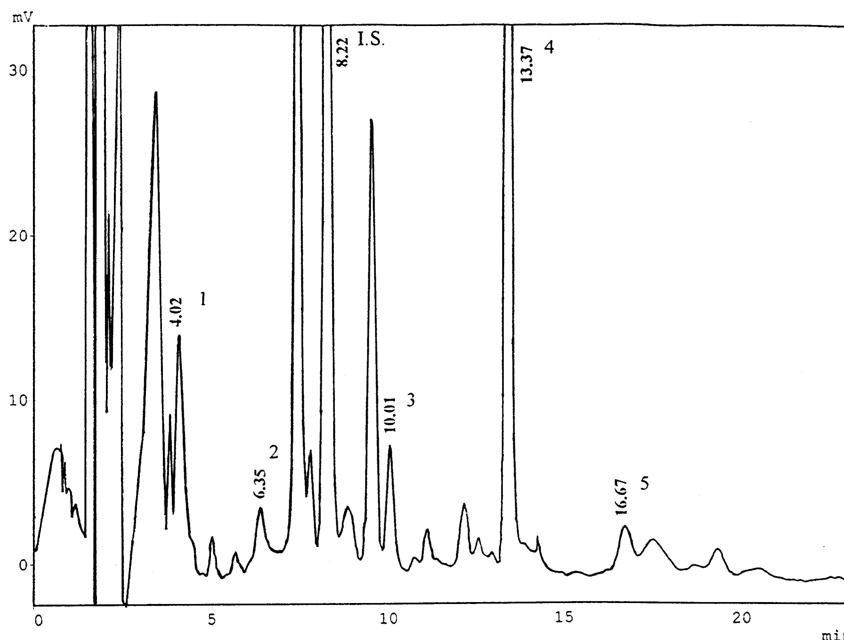


Fig. 2. Chromatogram of pooled urine sample. Peaks, 1, NE; 2, E; 3, NM; 4, DA; 5, MN; and IS, internal standard; DHBA.

Table 1
The important parameters for the calibration curves[a]

Analytes ($n=3$)	$y=ax+b$	r^2	RSD_a (%)	RSD_b (%)
NE	$0.0015x-0.130$	0.9982	2.2	8.5
E	$0.0008x+0.0812$	0.9943	8.6	1.6
DA	$0.0014x-0.0955$	0.9906	0.7	9.2
NM	$0.0006x-0.0588$	0.9948	0.1	6.0
MN	$0.0015x+0.0299$	0.9989	10.7	8.4

[a] a, Slope; b, intercept; r^2, r-squared; RSD_a, relative standard deviation of the slope; RSD_b, relative standard deviation of the intercept.

resin and thus did not interfere with the assay [20]. A linear relationship of peak area ratio over concentration range of 40–4000 nmol l^{-1} for each of the five analytes was obtained. The validating parameters of each calibration curve, such as slope (a), intercept (b), r-squared (r^2), relative standard deviation of the slope (RSD_a) and intercept (RSD_b), are presented in Table 1. The within-day %coefficients of variation for NE, E, NM, DA and MN at 50 nmol l^{-1} were 3.18, 9.71, 4.51, 2.65 and 2.58 and the between-day coefficients of variation were 3.71, 8.09, 8.74, 8.18 and 12.97, respectively. The absolute recoveries (%, mean ± S.D.) of NE, E, DA, NM and MN at 50 and 400 nmol l^{-1} were 78.47 ± 6.99, 93.43 ± 5.64, 79.89 ± 3.07, 82.54 ± 6.99, 69.22 ± 4.66 and 84.73 ± 2.03, 83.43 ± 5.07, 85.01 ± 3.05, 79.97 ± 5.07, 74.13 ± 1.78, respectively ($n=5$ for each concentration). Daily calibrations between 40 and 800 nmol l^{-1} of extracted aqueous analytes were also linear ($r^2>0.99$, $n=2$ for each concentration). The LOD and LOQ of the catecholamines and metanephrines in urine samples were 12.0 and 40.0 nmol l^{-1}, respectively. The stability-indicating capability of the assay had already been verified in our previous study [21] by degradation of

NE, E, DA, NM, MN and DHBA in 1 M HCL, 1 M NaOH and 300 g l^{-1} hydrogen peroxide solutions at 90°C for 1 h. No interfering peaks were observed.

3.2. Stability profiles of catecholamines and metanephrines in aqueous samples

Stability profiles of NE, E, DA, NM and MN in aqueous samples stored at 10 and 30°C over 8 days were illustrated in Fig. 3. The data of the mean ± S.D. of the % remaining of the initial concentrations (day 0) of NE, E, DA, NM and MN on days 1, 3, 5 and 8 are presented in Table 2. A loss exceeding 10% of the initial concentration is considered to indicate instability of the compounds.

From Fig. 3 and Table 2, we observed that all analytes were unstable in unpreserved aqueous solution stored at 30°C. The mean percentages remaining on day 8 for NE, E, DA, NM and MN were found to be 66, 44, 15, 79 and 53, respectively. In unpreserved aqueous sample stored at 10°C, all compounds were found to be stable over 8 days. It was apparent that the stability of these compounds in unpreserved samples could be improved by storage at lower temperature. Table 2 also showed that all catecholamines and metanephrines were stable in the preserved samples stored at 10°C over 8 days, except for MN in the sample preserved with Na_2EDTA and sodium metabisulfite. In this part of the study, we confirmed that our assay was capable of detecting a decline in concentration when degradation occurred.

3.3. Stability profiles of catecholamines and metanephrines in urine samples

Stability profiles of NE, E, DA, NM and MN in urine samples stored at 10 and 30°C over 8 days were illustrated in Fig. 4. The % remaining of the initial concentrations (day 0) of NE, E, DA, NM and MN on days 1, 3, 5 and 8 are

Table 2
Stability of NE, E, DA, NM and MN in unpreserved and preserved aqueous samples stored at 10 and 30°C over 8 days[a]

Samples[b]	Temperature		Day			
			1	3	5	8
UP	10°C	NE	103 ± 3	104 ± 2	96 ± 6	92 ± 4
		E	98 ± 1	98 ± 0	101 ± 3	102 ± 4
		DA	97 ± 1	98 ± 3	91 ± 3	99 ± 2
		NM	91 ± 2	90 ± 1	95 ± 0	93 ± 3
		MN	90 ± 0	90 ± 0	91 ± 1	92 ± 1
UP	30°C	NE	70 ± 3	68 ± 1	68 ± 1	66 ± 3
		E	75 ± 4	76 ± 2	80 ± 6	44 ± 1
		DA	89 ± 5	73 ± 9	61 ± 0	15 ± 1
		NM	94 ± 8	88 ± 4	93 ± 1	79 ± 4
		MN	76 ± 3	68 ± 4	77 ± 2	53 ± 4
HP	10°C	NE	93 ± 1	92 ± 1	96 ± 3	90 ± 3
		E	96 ± 4	96 ± 2	98 ± 3	99 ± 4
		DA	99 ± 4	101 ± 1	93 ± 1	101 ± 2
		NM	98 ± 2	96 ± 1	105 ± 3	99 ± 1
		MN	97 ± 0	92 ± 0	92 ± 1	94 ± 1
EMP	10°C	NE	91 ± 1	93 ± 2	97 ± 2	92 ± 1
		E	95 ± 1	96 ± 1	97 ± 1	100 ± 2
		DA	98 ± 2	102 ± 2	92 ± 3	101 ± 3
		NM	94 ± 1	94 ± 2	103 ± 2	97 ± 5
		MN	74 ± 1	75 ± 1	83 ± 1	83 ± 1

[a] Data are mean ± S.D. of the ratios (%) of the concentrations to the mean concentrations on day 0 ($n = 3$).
[b] UP, unpreserved; HP, HCl preserved; EMP, Na_2EDTA and sodium metabisulfite preserved.

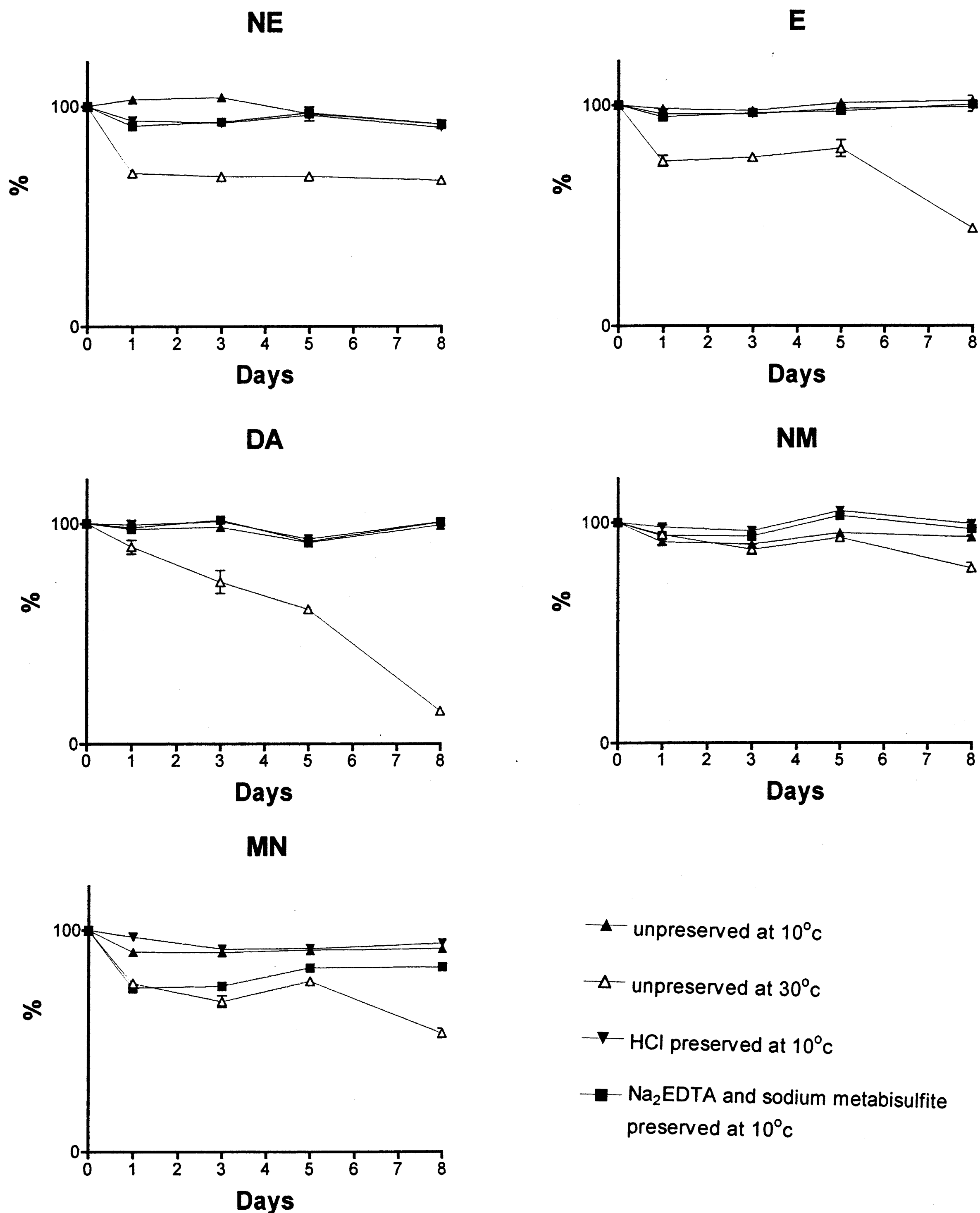


Fig. 3. Concentrations of NE, E, DA, NM and MN measured in unpreserved and preserved aqueous samples stored at 10 and 30°C over 8 days. Results are expressed as percentage of the mean concentrations at day 0.

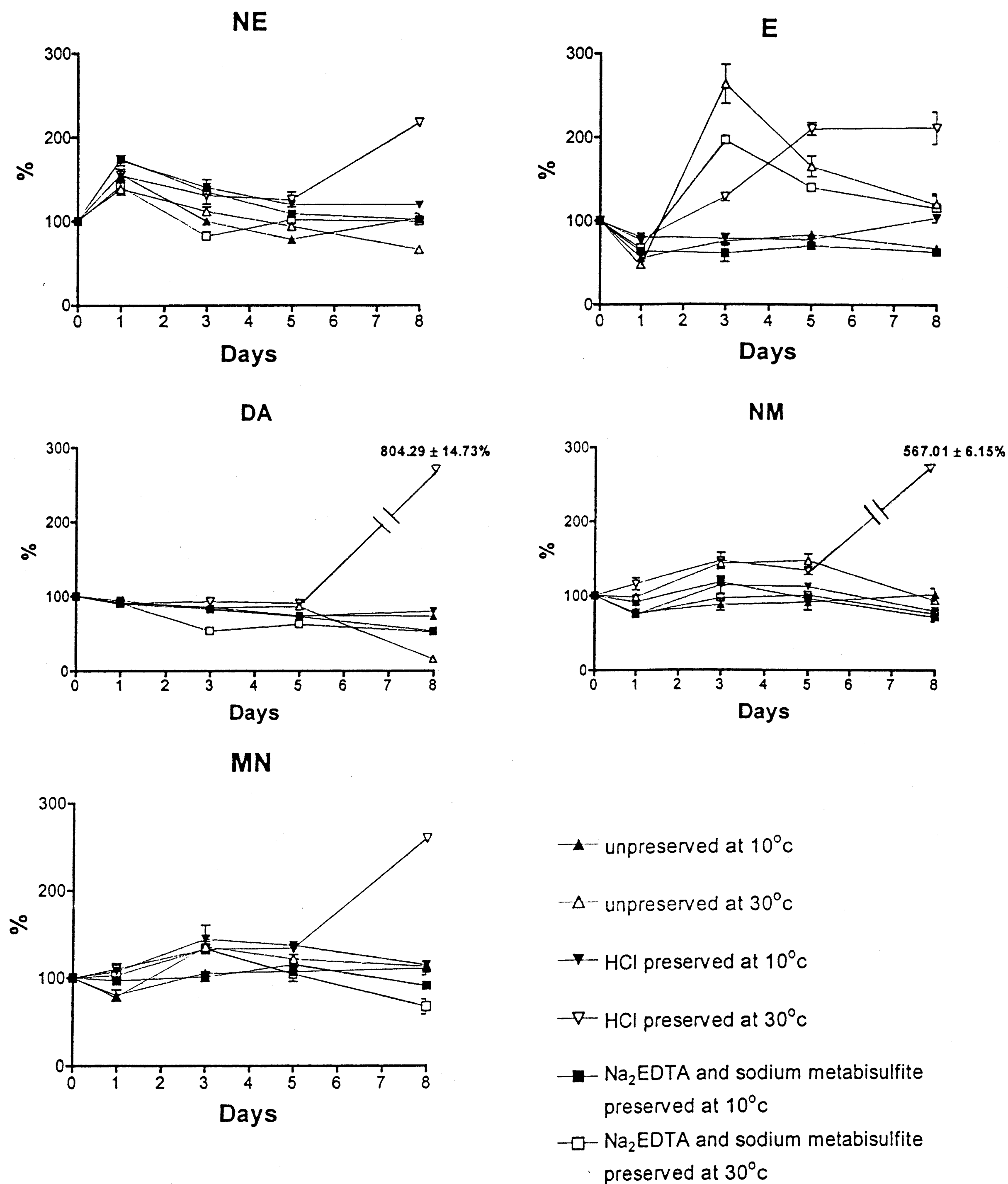


Fig. 4. Concentrations of NE, E, DA, NM and MN measured in unpreserved and preserved urine samples stored at 10 and 30°C over 8 days. Results are expressed as percentage of the mean concentrations at day 0.

Table 3
Stability of NE, E, DA, NM and MN in unpreserved and preserved urine samples stored at 10 and 30°C over 8 days[a]

Samples[b]	Temperature		Day			
			1	3	5	8
UP	10°C	NE	156 ± 11	100 ± 2	78 ± 2	104 ± 7
		E	56 ± 1	75 ± 0	83 ± 0	68 ± 5
		DA	92 ± 5	85 ± 0	74 ± 5	73 ± 1
		NM	77 ± 3	88 ± 11	91 ± 15	102 ± 12
		MN	80 ± 11	105 ± 3	107 ± 3	111 ± 11
UP	30°C	NE	139 ± 13	112 ± 7	94 ± 0	66 ± 2
		E	47 ± 2	263 ± 33	165 ± 17	121 ± 14
		DA	91 ± 1	84 ± 1	86 ± 1	16 ± 0
		NM	98 ± 6	143 ± 7	147 ± 13	94 ± 3
		MN	78 ± 2	135 ± 9	121 ± 7	113 ± 2
HP	10°C	NE	173 ± 11	141 ± 13	120 ± 4	120 ± 1
		E	81 ± 7	79 ± 8	78 ± 5	104 ± 2
		DA	90 ± 1	83 ± 0	74 ± 1	80 ± 1
		NM	75 ± 4	114 ± 16	112 ± 1	80 ± 3
		MN	107 ± 16	144 ± 23	137 ± 7	115 ± 2
HP	30°C	NE	155 ± 4	131 ± 15	126 ± 13	218 ± 6
		E	77 ± 8	129 ± 7	210 ± 11	212 ± 27
		DA	91 ± 1	93 ± 4	91 ± 1	804 ± 15
		NM	116 ± 14	146 ± 16	134 ± 8	567 ± 6
		MN	110 ± 1	133 ± 8	134 ± 2	260 ± 2
EMP	10°C	NE	175 ± 8	136 ± 3	109 ± 3	102 ± 0
		E	64 ± 4	61 ± 14	70 ± 4	63 ± 1
		DA	95 ± 1	82 ± 2	72 ± 1	53 ± 0
		NM	91 ± 13	118 ± 8	96 ± 2	71 ± 9
		MN	96 ± 2	101 ± 8	114 ± 10	91 ± 6
EMP	30°C	NE	142 ± 11	82 ± 0	101 ± 0	100 ± 0
		E	67 ± 7	197 ± 0	140 ± 4	116 ± 25
		DA	91 ± 1	53 ± 0	63 ± 0	52 ± 3
		NM	75 ± 7	97 ± 0	100 ± 2	76 ± 3
		MN	103 ± 5	132 ± 0	104 ± 12	67 ± 12

[a] Data are mean ± S.D. of the ratios (%) of the concentrations to the mean concentrations on day 0 ($n = 3$).
[b] UP, unpreserved; HP, HCl preserved; EMP, Na_2EDTA and sodium metabisulfite preserved.

summarized in Table 3. In comparison to the stable or declining profiles of these analytes in aqueous samples stored at 10 and 30°C, their profiles in urine samples were highly fluctuating (Fig. 4).

Stability data in Table 3 show that in general, these compounds were unstable in both unpreserved and preserved urine stored at 10 and 30°C which is in contrary to the findings of Boosman et al. [16] that unpreserved urine samples could be stable at room temperature (20°C) for 4 days without significant loss. In our study, substantial changes in concentrations of these compounds were visible even on day 1 in both unpreserved and preserved urine samples stored at 10 and 30°C. This indicates that storing urine samples at room temperature could be detrimental to their stability. This is in agreement to the recommendation by Miki and Sudo [14] that for the analysis of catecholamines, urine samples whether preserved or unpreserved, should be frozen as soon as possible.

Data in Table 3 show that concentrations of NE were invariably greater than their respective concentrations on day 0 despite of the different preserving and storage conditions. These initial

increases were followed by continuous decline in its concentrations. With the exception of the unpreserved samples stored at 10°C, E, NM, and MN all showed substantial increases in concentrations during the storage period, often after an initial stable or decline in concentrations on day 1, whereas DA displayed consistent declining concentration under most of the preserving and storage conditions. One possible process accounting for this observation would be the deconjugation of CA-S and MN-S in urine samples during storage at 10 and 30°C. The measured urinary concentrations of these compounds over the storage period could be affected by the combined effects of degradation of the free amines and deconjugation of their sulfoconjugates.

DA did not display any increases in concentrations under most preserving and storage conditions over the 8 days. DA concentration only increased on day 8 for the HCL preserved urine sample stored at 30°C. One possible explanation was that deconjugation of DA-S occurred but not as rapidly as degradation of the free DA. This explanation was partially supported by results of unpreserved aqueous sample stored at 30°C (Fig. 3 and Table 2). In these aqueous samples, the mean percentages remaining on day 8 for NE, E, DA, NM and MN were found to be 66, 44, 15, 79 and 53, respectively. This indicated that DA degraded the most rapidly in unpreserved aqueous sample stored at 30°C. The increase in DA concentration observed in HCL preserved urine (Fig. 4) could be due to acid hydrolysis of DA-S after prolonged storage at 30°C.

Table 3 shows that the largest increases in catecholamine and metanephrine concentrations occurred in the acid preserved urine samples followed by the Na_2EDTA and sodium metabisulfite preserved samples. The unpreserved samples showed the least increases in their levels. The increases were especially higher in samples stored at 30°C. This suggested that preservation had no effect in impeding the deconjugation process. The propensity of CA-S and MN-S to undergo deconjugation in HCL preserved urine could be due to acid hydrolysis of the sulfate ester linkages. This hydrolysis process if occurred, would be accentuated at 30°C as compared to 10°C.

3.4. Extent of sulfoconjugation in spot urine samples

Results of the hydrolysis study of urine samples collected from four human subjects were presented in Table 4. From the results of the hydrolysis of aqueous samples, it was found that the degradation of NE, E, DA, NM and MN was minimal. Hence, no correction for degradation was needed for the data of the hydrolyzed urine samples. The data obtained for the urine samples were comparable to those obtained in other studies [11,20]. From Table 4, we observed that urine collected from male subject that underwent exercise contained higher amounts of catecholamines and metanephrines. Urine sample from hypertensive subject did not contain excessive amounts of these compounds, as not all hypertensive patients have high catecholamines and metanephrines in their bodies. Spot urine sample may also not reflect the concentrations in 24 h urine collection. Results from this part of the study confirmed that sulfoconjugation of NE, E, DA, NM and MN in urine was appreciable and deconjugation of these CA-S and MN-S was capable of increasing the detected amount of free urinary amines significantly.

3.5. Stability profiles of catecholamines and metanephrines at −80°C

Results of stability study of catecholamines and metanephrines in unpreserved urine and aqueous samples stored at −80°C for 29 days were summarized in Table 5. It was clear that NE, E, DA, NM and MN were stable (variations within 10% of the mean concentrations on day 0) in both urine and aqueous samples over 22 days. The stability profiles of these compounds in urine and aqueous samples stored at −80°C were comparable. From these results, we confirmed that deconjugation of urinary CA-S and MN-S and degradation of the free amines were minimized at extreme low temperature (−80°C) for at least 22 days. However, on day 29, it started to show some discrepancies in the NE and NM concentrations in the urinary and aqueous samples. This means that degradation and deconjugation could occur at that time.

4. Conclusion

In this study, the detected levels of catecholamines and metanephrines in biological samples were found to be affected by the simultaneous degradation of these compounds and deconjugation of their sulfoconjugates, when these samples were not stored and preserved prop-

Table 4
Free, total levels and percentages sulfoconjugation of urinary NE, E, DA, NM and MN in four human subjects

Subjects		Hypertensive[a]	Exercised[b]	Healthy	Healthy
Age (years)		59	27	52	27
Sex[e]		M	M	F	F
NE	Free[c]	161	1138	238	140
	Total[c]	408	2006	549	418
	%[d]	61	43	57	67
E	Free[c]	52	245	ND[f]	ND[f]
	Total[c]	59	227	44	57
	%	12	UD[g]	UD[g]	UD[g]
DA	Free[c]	1079	4332	1176	2294
	Total[c]	2865	7502	2035	6419
	%	62	42	42	64
NM	Free[c]	173	1288	434	421
	Total[c]	1405	6990	1663	3633
	%	88	82	74	88
MN	Free[c]	33	131	33	92
	Total[c]	132	364	65	214
	%	75	64	49	57

[a] Subject has essential hypertension for more than 5 years.
[b] Subject exercised for 1 h prior to urine collection.
[c] Results are expressed in nmol l^{-1}.
[d] Percentage of sulfoconjugation is calculated as (total − free)/total multiplies by 100%.
[e] M, male; F, female.
[f] ND, not detected.
[g] UD, undefined.

Table 5
Stability of NE, E, DA, NM and MN in unpreserved urine and aqueous samples stored at −80°C over 29 days[a]

Samples		Day 1	Day 3	Day 5	Day 8	Day 15	Day 22	Day 29
Urine	NE	100 ± 2	100 ± 3	100 ± 13	109 ± 6	96 ± 7	95 ± 6	95 ± 0
	E	102 ± 0	95 ± 8	98 ± 9	103 ± 4	96 ± 5	97 ± 0	98 ± 0
	DA	104 ± 0	101 ± 0	103 ± 0	103 ± 1	98 ± 2	105 ± 2	100 ± 6
	NM	104 ± 6	92 ± 3	103 ± 0	106 ± 1	95 ± 5	96 ± 4	103 ± 0
	MN	101 ± 6	102 ± 0	97 ± 11	104 ± 8	101 ± 0	99 ± 1	96 ± 5
Aqueous	NE	100 ± 1	97 ± 3	92 ± 1	96 ± 1	91 ± 4	104 ± 2	84 ± 0[b]
	E	100 ± 1	95 ± 5	97 ± 1	95 ± 6	98 ± 4	91 ± 3	96 ± 3
	DA	100 ± 0	98 ± 5	95 ± 0	94 ± 2	103 ± 0	98 ± 6	99 ± 3
	NM	98 ± 1	98 ± 12	102 ± 2	98 ± 7	94 ± 0	94 ± 3	70 ± 8[b]
	MN	106 ± 2	107 ± 4	106 ± 1	95 ± 3	102 ± 1	103 ± 4	108 ± 3

[a] Data are mean ± S.D. of the ratios (%) of the concentrations to the mean concentrations on day 0 ($n = 2$).
[b] Decreases greater than 10% of mean concentrations on day 0 indicate instability of compound.

erly. Deconjugation of urinary CA-S and MN-S could occur during the first 3 days of storage at 10 and 30°C in both unpreserved and preserved urine samples. Addition of preservatives (HCl or Na_2EDTA and sodium metabisulfite) was found to have no effect in impeding the deconjugation process. Instead, deconjugation was accentuated by the presence of HCl (3 M, 5 ml l^{-1}) as a preservative in the sample and at higher temperature (30°C). However, storing the samples at extreme low temperature could minimize the deconjugation process. Free catecholamine and metanephrine concentrations were found to be stable for at least 3 weeks in both unpreserved urine and aqueous samples stored at −80°C. From this, we concluded that deconjugation of CA-S and MN-S and degradation of their free amines did not occur during this period in the unpreserved urine samples stored at −80°C.

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