



# A rapid and sensitive fluorescence method for detecting urine formaldehyde in patients with Alzheimer's disease

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## Abstract

**Background:** Morning urine formaldehyde concentrations could predict the severe degree of dementia in patients with post-stroke dementia and Alzheimer's disease. However, the routinely available technique of high-performance liquid chromatography (HPLC) for detecting urine formaldehyde requires expensive and sophisticated equipment.

**Methods:** We established a fluorescence spectrophotometric method by using a formaldehyde-specific fluorescent probe-NaFA ( $\lambda_{ex/em} = 430/543$  nm). As a standard reference method, the same batch of urine samples was analysed by HPLC with a fluorescence detector ( $\lambda_{ex/em} = 346/422$  nm). Then we compared the limits of detection and the limits of quantization detected by these two methods and addressed the relationship between urine formaldehyde and human cognitive ability. The Mini-Mental State Examination (MMSE), Clinical Dementia Rating and Activities of Daily Living scale were used to evaluate cognition function in 30 Alzheimer's disease patients and 52 healthy age-matched controls.

**Results:** Limits of detection and limits of quantization (1.27 and 2.48  $\mu\text{M}$ ) of the NaFA probe method were more accurate than Fluo-HPLC (1.52 and 2.91  $\mu\text{M}$ ). There was no difference in the detected formaldehyde values within day and day-to-day. Notably, only 3/82 urine formaldehyde concentrations detected by NaFA probe were below zero, while 12/82 of the values analysed by Fluo-HPLC were abnormal. More importantly, there were negatively correlated between urine formaldehyde concentrations detected by NaFA probe and MMSE scores, but positively correlated with Clinical Dementia Rating scores in Alzheimer's disease patients.

**Conclusions:** This detecting urine formaldehyde method by NaFA probe was more rapid, sensitive and accurate than Fluo-HPLC.

## Keywords

Formaldehyde, NaFA, spectrophotometry, high-performance liquid chromatography, dementia, Alzheimer's disease

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## Introduction

Exogenous gaseous formaldehyde (FA), notoriously known as an indoor air pollutant, induces animal memory loss<sup>1</sup> and human cognitive impairments.<sup>2</sup> Surprisingly, endogenous FA has been found to be existing in all invertebrate and vertebrate cells.<sup>3</sup> Normal brain FA concentration in healthy mice, rats and human is about 300  $\mu\text{M}$ , which can be detected by gas chromatography/mass spectrometry (GC/MS),<sup>4</sup> or high-performance liquid chromatography with a fluorescent probe (Fluo-HPLC).<sup>5</sup> Notably, an abnormal high concentration of hippocampal FA ( $\sim 500 \mu\text{M}$ ) has been found in the patients with Alzheimer's disease (AD), APP/PSI transgenic AD-like model mice and the senescence-accelerated mouse SAMP.<sup>6</sup> Injection of 500  $\mu\text{M}$  FA indeed impairs memory in mice and rats.<sup>7</sup> Unsurprisingly, excess FA has strong neurotoxicity<sup>8</sup> because it can accelerate  $A\beta$  aggregation and Tau hyperphosphorylation in normal adult mice and monkeys<sup>9, 10</sup> and lead to cognitive decline.<sup>11</sup> In clinic, morning urine FA concentrations are negatively correlated with cognitive ability in elder people,<sup>12</sup> especially in patients with Alzheimer's disease (AD).<sup>6</sup> A cross-sectional survey in 577 participants has found that urine FA with cut-off (41.8  $\mu\text{M}$ ) could predict the severe degree of dementia in patients with poststroke dementia (PSD) or AD.<sup>13</sup>

In recent decades, different kinds of methods have been established to detect urine FA concentrations. For example, the average concentration of human FA urine analysed by headspace gas chromatography (GC) is  $\sim 33 \mu\text{M}$ .<sup>14</sup> This result is similar to the value ( $\sim 29 \mu\text{M}$ ) detected by Fluo-HPLC, which is based on the fact that ampicillin has chemical reaction with FA.<sup>13</sup> Similarly, urine FA concentration in rats analysed by HPLC with ultraviolet (UV) detector is approximately 32  $\mu\text{M}$ .<sup>15</sup> A more sensitive radiometric method by using <sup>14</sup>C-dimedone reagent has been established, and the range of FA values are about 10–20  $\mu\text{M}$ .<sup>16</sup> These data indicate that urine FA concentrations in healthy adult humans are less than 40  $\mu\text{M}$  (the pathological concentration in AD patients). Although these existing approaches provide accurate and sensitive detection of FA, several disadvantages, such as sophisticated experimental procedures and noxious analytical reagents, have hindered their clinical and practical applications. Therefore, a simple, sensitive and efficient method for determining trace amounts of urine FA is urgently needed.

Here, we established a highly sensitive and selective spectrophotometric method for detecting urine FA at room temperature by using NaFA probe because it can specifically react with FA to produce a fluorescent derivant ( $\lambda_{\text{ex/em}} = 430/543 \text{ nm}$ ).

## Materials and methods

### Ethics

This clinical investigation (2014SY39) was approved by the Ethics Committee at the Capital Medical University, China.

### Participants

The study was registered at the Chinese Clinical Trial Registry (<http://www.chictr.org/cn>, Unique Identifier: ChiCTR-OOC-14005576), and conducted between March 2008 and December 2014. We recruited participants from representative regions in Beijing, China. The mean age of all individuals in this cross-sectional survey was  $78.69 \pm 2.58$  years ( $n = 82$ ). Participants who refused to provide urine samples, or had a life-threatening illness, or were unable to participate in the assessment, were excluded from the entire survey. Informed consent was obtained from each participant directly or indirectly from his or her guardian.

### Clinical evaluation

The cognitive status of patients/participants was assessed by neurologists using the Activities of Daily Living (ADL),<sup>17</sup> Clinical Dementia Rating (CDR)<sup>18</sup> and Mini Mental State Examination (MMSE).<sup>19</sup> A MMSE score  $\leq 20$  (adjusted for education level of the participants from rural regions) was defined to be cognitive impairment. The MMSE is widely applied to assess the cognitive ability of patients suffering from memory decline.<sup>19</sup> PSD and AD were distinguished and diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders (fourth edition) revised (DSM-IV-R) criteria, as described previously.<sup>20</sup> Information on medical history and medications for each participant were obtained from primary health-care records, or provided by each participant or his or her guardian.

### Morning urine samples

The morning urine samples from participants were collected and immediately placed on ice, before being stored at  $-70^\circ\text{C}$  until analysis. After centrifugation ( $8000 \times g$ ,  $4^\circ\text{C}$ , 10 min), supernatant fractions of urine were subjected to urine FA analysis.

### Chemical reagents

FA-specific fluorescent probe-NaFA was provided by Prof. Weiyang Lin (Institute of Fluorescent Probes for Biological Imaging, University of Jinan, China). Ampicillin (D-(2)-a-aminobenzylpenicillin anhydrous), dimethyl sulfoxide (DMSO), 37% FA solution and

trichloroacetic acid (TCA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other reagents were of analytical grade. All solvents were of HPLC grade (Tedia, Fairfield, OH, USA) and deionized water was obtained from the Milli-Q system.

### Detection of urine FA by fluorescent spectrophotometer

To determine the optimal time of derivative reaction between FA and FA probe-NaFA at room temperature, seven 100- $\mu$ L aliquots of different concentrations of FA standard solutions (1, 5, 10, 50, 100, 500 and 1000  $\mu$ M, pH at 7.4, respectively) were added to seven vials in order to prepare a series of calibration standards. In addition, 800  $\mu$ L of PBS with pH at 7.4 was pipetted into a separate vial after the aliquots of 100  $\mu$ L 20  $\mu$ M NaFA solution with pH at 7.4 were added to each of these seven vials. These mixtures were pipetted into 96-well plates and incubated at room temperature for 0, 30, 60 and 120 min, respectively. Then the fluorescent intensities of the derivant between standard FA and NaFA, between urine FA and NaFA were quantified by a fluorescent spectrophotometer at  $\lambda_{ex/em} = 430/543$  nm (Multi-Mode Microplate Reader, SpectraMax i3, Molecular Devices, California, USA), respectively (Supplementary Figure 1).

The calibration curves which covered the FA concentration range of 1–1000  $\mu$ M were prepared. For routine analysis, a one-point calibration in duplicate was prepared daily and used for quantitative calculation of urine samples.

### Detection of urine FA by Fluo-HPLC

As a standard method reference, the same batch of urine samples was routinely analysed by using Fluo-HPLC ( $\lambda_{ex/em} = 346/422$  nm) as described previously.<sup>13, 21</sup> Briefly, 200  $\mu$ L human urine was pipetted into a 2-mL glass vial, to which 800  $\mu$ L water, 100  $\mu$ L ampicillin solution (2.5 mg/mL, in water) and 250  $\mu$ L TCA (20%, w/v, in water) were added. The vial was heated in a 90°C water bath for 1 h. After cooling to room temperature, the content of the vial was transferred to a 10-mL glass centrifuge tube. The vial was rinsed with 1 mL diethyl ether twice and also transferred to the centrifuge tube. About 0.5 g sodium chloride (NaCl) was added to precipitation urine proteins. After centrifugation, the upper layer was extracted with another 1 mL diethyl ether. The diethyl ether was evaporated to dryness with a gentle stream of nitrogen. The residue was redissolved in 500  $\mu$ L acetonitrile–water (50:50), and was ready for Fluo-HPLC analysis (Agilent HP1100, USA) (Supplementary Figure 1).

### Variations of within-day and day-to-day

Urine samples ( $n = 10$ ) were analysed for fluorescent intensity on day 1 to evaluate the variation in within-day FA concentrations at 0, 30, 60 and 120 min, respectively. Another group was used to evaluate the variation in within day and day-to-day of FA concentrations, respectively.

### Data analysis

Statistical analyses were performed using IBM SPSS software for Windows (version 19.0; SPSS Inc., Chicago, IL, USA). Graphs were generated using GraphPad Prism version 5.01 (GraphPad Software Inc., San Diego, CA, USA). The clinical characteristics and urine formaldehyde concentrations were compared using the  $\chi^2$  statistic for categorical variables and analysis of variance for continuous variables. The concentrations of formaldehyde in urine samples from AD patients and age-matched controls were compared using Student's *t*-test. Differences between the groups were considered statistically significant when the *P* value was less than 0.05.

## Results

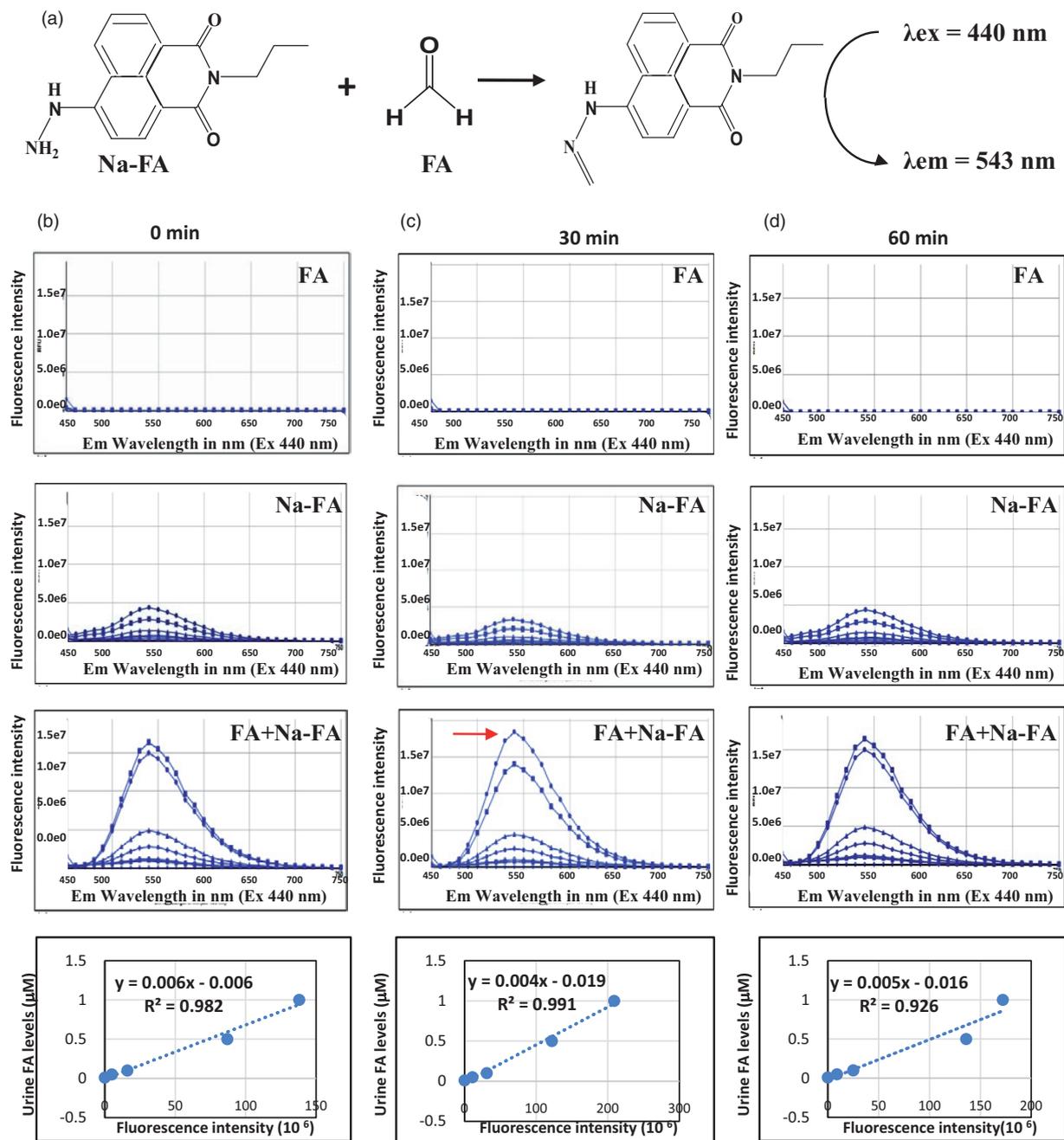
### Optimization of the reaction conditions

To examine the sensitivity of the NaFA probe method, we explored the experimental parameters' reaction time at room temperature ( $25 \pm 1^\circ\text{C}$ ). We found that the highest fluorescent intensity was at 30 min and then gradually declined at 1 h after the standard FA was mixed with NaFA probe at room temperature (Figure 1(a) to (d)). The derivant between NaFA and FA can be detected by fluorescent spectrophotometry at  $\lambda_{ex/em} = 430/543$  nm.<sup>22</sup> Therefore, reaction times at 30 min and room temperature at  $25^\circ\text{C}$  were the optimal conditions for the derivative reaction.

As a standard reference method,<sup>5,13</sup> the available approach of Fluo-HPLC for detecting urine FA was carried out in this study. We found that ampicillin had chemical reaction with FA and formed a derivant, which can be detected by HPLC with a fluorescence detector ( $\lambda_{ex/em} = 346/422$  nm). There was a hypothetical standard curve between FA and area of fluorescent peaks ( $R^2 = 0.996$ ) (Figure 2(a) to (e)).

### Limits of detection and limits of quantization of methods

Next we tested the limits of detection (LOD) and the limits of quantization (LOQ) of urine FA, which were analysed by these two methods. In routine analysis, a one-point calibration in duplicate could be used instead of a calibration curve, but a new calibration curve was



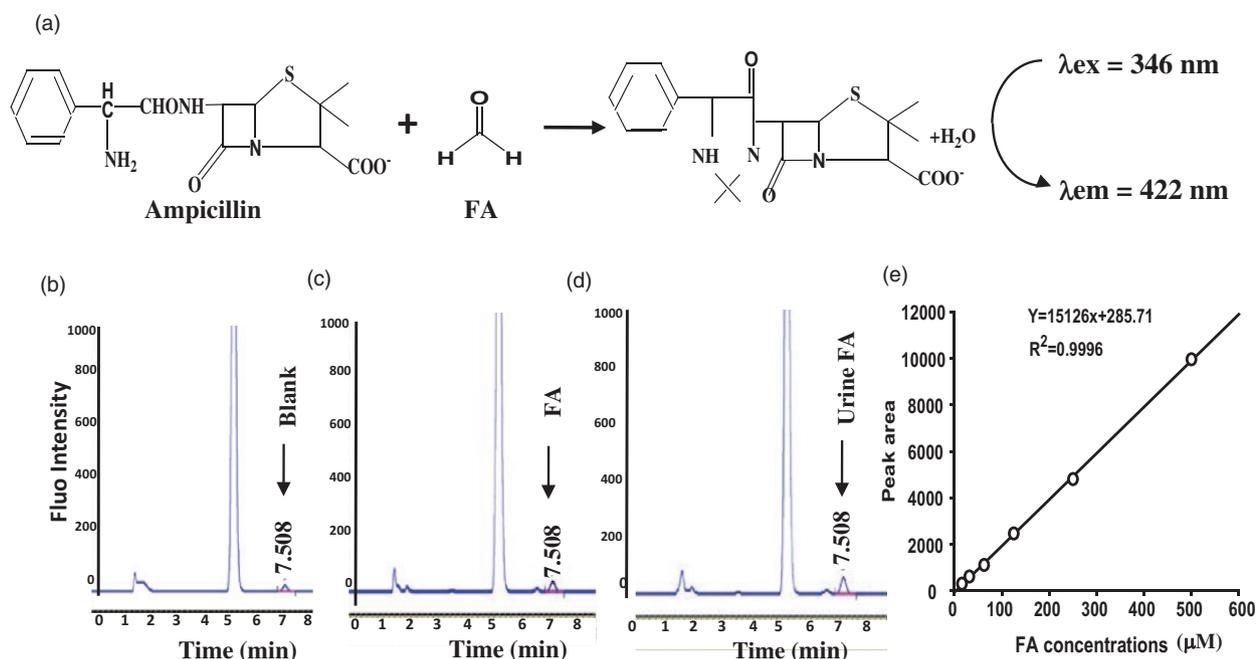
**Figure 1.** The standard curves of FA derivation with NaFA (a FA-specific fluorescent probe) analysed by fluorescence spectrophotometry. (a) The derivant between FA and NaFA detected at  $\lambda_{ex}/\lambda_{em} = 430/543 \text{ nm}$  and (b) to (d) The chemical reaction time and standard curves between FA and NaFA at 0, 30, 60 and 120 min, respectively. FA: formaldehyde.

prepared to check the instrumental system in case of any deviation. The LOD and LOQ were calculated from the confidence intervals of the regression line of the calibration line following the method as previously described.<sup>23–25</sup> Our results showed that the LOD and LOQ of using FA probe method were 1.27 and 2.48  $\mu\text{M}$ , while 1.52 and 2.91  $\mu\text{M}$  for Fluo-HPLC method (Table 1). The average recoveries were 97.3% and

98.1% with relative standard deviations (RSDs,  $<8\%$ ). These data indicate that this NaFA probe method is more accurate than Fluo-HPLC.

#### Repeatability and stability of comparative methods

Ten replicates of urine samples were analysed at various time points in a day to evaluate within-day



**Figure 2.** The standard curves of FA derivatization with ampicillin analysed by Fluo-HPLC. (a) The derivant between FA and ampicillin detected at  $\lambda_{ex}/\lambda_{em} = 346/422$  nm. (b) to (d). The HPLC peak of blank control, FA and urine FA, respectively. Retention time: 7.508 min and (e) Standard curves between FA and peak area of fluorescence intensity. FA: formaldehyde.

**Table 1.** LOD, LOQ, RSD and urine FA concentrations detected by using FA probe and Fluo-HPLC methods within a day at different time points, respectively.

Test items	0 min	30 min	1 h	2 h	Mean $\pm$ S.D.	Recovery (%)	RSD (%)	LOD ( $\mu$ M)	LQD ( $\mu$ M)
FA probe-urine FA	94.52	96.85	92.17	91.03	93.64 $\pm$ 6.26	97.3	6.68	1.27	2.48
HPLC-urine FA	52.49	53.33	53.58	54.06	53.36 $\pm$ 3.85	98.1	7.22	1.52	2.91

Note: Data in means ( $n = 10$ ). No significant difference among the values detected by these two methods within a day ( $P > 0.05$ ), respectively. FA: formaldehyde; LOD: limits of detection; LOQ: limits of quantization; RSD: relative standard deviations; HPLC: high-performance liquid chromatography.

**Table 2.** Urine FA concentrations detected by using FA probe and HPLC methods at different days, respectively.

Test items	One day	Two days	Mean	S.D.	Recovery (%)	RSD (%)
FA probe-Urine FA ( $\mu$ M)	96.85	103.87	100.36	7.07	97.6	7.04
HPLC-Urine FA ( $\mu$ M)	53.42	56.79	55.10	3.73	96.2	6.76

Note: Data in means ( $n = 10$ ). No significant difference among the values detected by these two methods day-to-day ( $P > 0.05$ ), respectively. FA: formaldehyde; RSD: relative standard deviations; HPLC: high-performance liquid chromatography.

variations. The same samples of urines were also analysed on different days to evaluate day-to-day variations. The data of variations of within-day are summarized in Table 1. These data indicate that there is no difference in the concentrations of FA in urine samples within a day ( $P > 0.05$ ). The results of variation of day-to-day showed that the average relative

standard deviations were 7.07 for FA probe method and 3.73 for Fluo-HPLC method (Table 2). The average recoveries were 97.6% and 96.2% with RSDs ( $< 8\%$ ). These data indicate that these two methods can effectively and steadily determine urine FA concentrations. However, the method of NaFA probe was more sensitive than Fluo-HPLC because the average

concentrations of urine FA detected by prior approach ( $93.64 \pm 10.29 \mu\text{M}$ ) were higher than the latter method ( $53.36 \pm 8.32 \mu\text{M}$ ) (Table 2).

### Relationship between urine FA and human cognitive ability

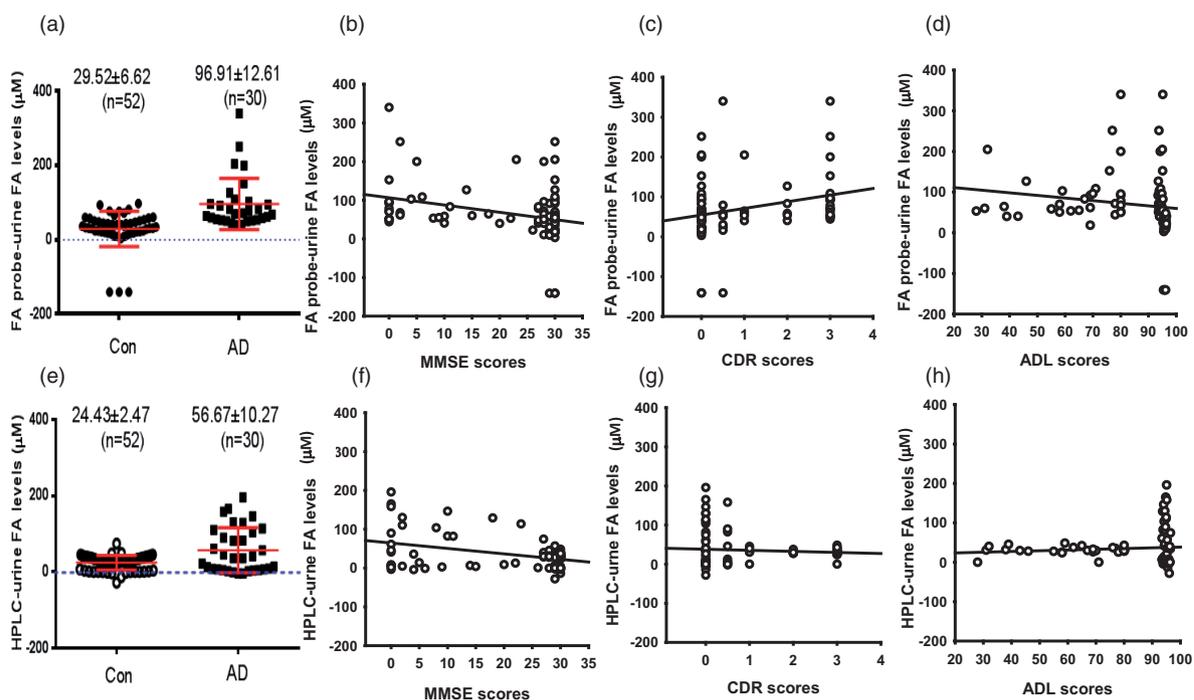
To address whether urine FA predicts the degree of dementia, we evaluated human cognitive ability by using the Mini-Mental State Examination (MMSE), CDR and ADL in 30 AD patients and

52 age-matched controls, and analysed urine FA by these two methods (Table 3). The results showed that urine FA concentrations in AD patients ( $96.91 \pm 12.61 \mu\text{M}$ ) analysed by using FA probe were obviously higher than age-matched controls ( $29.52 \pm 6.62 \mu\text{M}$ ,  $P < 0.01$ ) (Figure 3(a)). More importantly, using the NaFA method, urine FA concentrations were negatively correlated with MMSE scores but positively correlated with CDR scores, and not relative with ADL in 82 participants (Figure 3(b) to (c)). As a standard reference method, urine FA concentrations

**Table 3.** Comparative urine FA concentrations detected by using FA probe and Fluo-HPLC methods in patients with Alzheimer's disease and age-matched controls, respectively.

Test items/groups	Controls (n = 52)	AD (n = 30)	P
Age (years old)	$77.01 \pm 3.45$	$80.02 \pm 2.36$	$>0.05$
Sex (male/female)	21/31	12/18	$>0.05$
Education (years)	$8.65 \pm 2.76$	$8.38 \pm 2.82$	$>0.05$
MMSE scores	$29.19 \pm 2.15$	$6.16 \pm 1.37$	$<0.01$
CDR scores	$0.08 \pm 0.03$	$2.50 \pm 0.14$	$<0.01$
ADL scores	$94.84 \pm 2.37$	$63.63 \pm 3.13$	$<0.01$
FA probe-urine FA ( $\mu\text{M}$ )	$29.52 \pm 6.62$	$96.91 \pm 12.61$	$<0.01$
HPLC-urine FA ( $\mu\text{M}$ )	$24.43 \pm 2.47$	$56.67 \pm 10.27$	$<0.01$

FA: formaldehyde; AD: Alzheimer's disease; MMSE: Mini-Mental State Examination; CDR: Clinical Dementia Rating; ADL: Activities of Daily Living.



**Figure 3.** The relationship between the score of MMSE/CDR/ADL and urine FA concentration analysed by these two methods. (a to d) Urine FA detected by using FA probe (a), the relationship between FA and MMSE (b), CDR (c), ADL (d), respectively. (e to h) Urine FA detected by using Fluo-HPLC, (e) the relationship between FA and MMSE (f), CDR (g), ADL (h), respectively. FA: formaldehyde; MMSE: mini-mental state examination; CDR: Clinical Dementia Rating; ADL: Activities of Daily Living scale.

detected by Fluo-HPLC were  $56.67 \pm 10.27 \mu\text{M}$  in AD patients and  $24.43 \pm 2.47 \mu\text{M}$  in healthy controls (Figure 3(e)). However, the correlation coefficient between MMSE/CDR and urine FA detected by Fluo-HPLC was lesser than that analysed by NaFA probe (Figure 3(f) to (h)). These data indicate that the method of NaFA probe is more suitable to analyse urine FA.

## Discussion

Compared with the methods of GC/MS, Fluo-HPLC and UV-HPLC, the technique of NaFA probe for detecting urine FA was relatively simple and rapid. It is a promising approach for clinical diagnosis and medicine monitoring in AD patients.

The accuracy, repeatability and stability of the new method are critical for clinical biochemical investigations. In this study, LOD and LOQ ( $1.27$  and  $2.48 \mu\text{M}$ ) of NaFA probe method were more accurate than Fluo-HPLC ( $1.52$  and  $2.91 \mu\text{M}$ ). There was no difference in urine FA concentrations within day and day-to-day. Only 3/82 urine FA concentrations detected by NaFA probe were below zero, while 12/82 of the values analysed by Fluo-HPLC were abnormal. Each urine FA value in AD patients (30/30) detected by NaFA probe was higher than the average concentrations in healthy controls ( $29.52 \pm 6.62 \mu\text{M}$ ) (Figure 3(a)). However, only 19/30 of urine FA concentrations analysed by Fluo-HPLC were higher than the average concentrations in healthy controls ( $24.43 \pm 2.47 \mu\text{M}$ ) (Figure 3(e)). More importantly, the correlation coefficient between MMSE/CDR and urine FA detected by NaFA probe was higher than Fluo-HPLC. These data indicate that the method of NaFA probe is more accurate than Fluo-HPLC.

The specificity of the derivant reagent determines the accuracy of these two methods of NaFA probe and Fluo-HPLC. The fluorescent probe of NaFA has been developed recently,<sup>26</sup> and it has a substantial high specificity and sensitivity to FA, because NaFA did not have chemical reaction with a lot of relevant analytes, such as PBS, glyoxal, methylglyoxal, sodium pyruvate, p-hydroxybenzaldehyde, trichloroacetaldehyde, acetaldehyde, 4-nitro-benzaldehyde, acetone, NaClO, H<sub>2</sub>O<sub>2</sub>, tert-butyl hydroperoxide, TBHP, nitric oxide (NO); CaCl<sub>2</sub>, MgCl<sub>2</sub>, Na<sub>2</sub>SO<sub>3</sub>, NaNO<sub>2</sub>, NaHSO<sub>3</sub>, NaHS, L-Arg, GSH, L-Cys, DL-Hcy, D-Phe, N-acetylglycine and N-acetyl-cysteine. In this study, we found that the selectivity and sensitivity of Na-FA probe to urine FA were similar to previous report.<sup>26</sup> For example, these unsaturated aldehydes at  $50 \mu\text{M}$ , including glyoxal, methylglyoxal, acetaldehyde, trichloroacetaldehyde, 4-nitro-benzaldehyde,

p-hydroxybenzaldehyde, had extremely lower sensitivity than FA (Supplementary Figure 2(a) and (b)). The average FA concentration of these aldehydes detected by NaFA probe was about  $1.5 \mu\text{M}$ , which was much less than urine FA (about  $30 \mu\text{M}$ ,  $P < 0.01$ ). Meanwhile, we found that these FA scavengers at  $10 \mu\text{M}$ , including L-Cys,<sup>27</sup> N-acetyl-cysteine<sup>28</sup> and NaHSO<sub>3</sub>,<sup>26</sup> could reduce urine FA concentrations (Supplementary Figure 2(b)). However, urine components contain only  $\sim 0.16 \mu\text{M}$  L-cys and almost nothing of N-acetyl-cysteine and NaHSO<sub>3</sub>. These data indicate that the above-mentioned compounds do not disturb the sensitivity of urine FA. More importantly, the derivant between FA and NaFA can be specifically detected by fluorescent spectrophotometry at  $\lambda_{\text{ex/em}}$ :  $440/543 \text{ nm}$ .<sup>22</sup> Although the method of Fluo-HPLC at  $\lambda_{\text{ex/em}}$ :  $346/422 \text{ nm}$  is based on that the derivant between ampicillin and FA, the specificity of ampicillin via above relevant analytes was not examined.<sup>5</sup> In this study, LOD and LOQ of the NaFA probe method were more sensitive than Fluo-HPLC, suggesting that this method of NaFA probe has relatively good accuracy and sensitivity.

Another critical question was whether the pH values of urine affect the detected FA concentrations by using this NaFA-probe method. First, there was no statistical difference in the average pH values of urine samples of six AD patients and six age-matched controls selected from sample bank randomly, were  $6.76 \pm 0.24$  and  $6.84 \pm 0.39$ , respectively (Supplementary Figure 3(a)). Second, to rule out the effects of pH on urine FA concentrations, we artificially adjusted the pH values to 6.2, 6.8 and 7 of these randomly selected 12 samples, respectively, and then analysed urine FA concentration. Notably, under the same condition of these urine samples at the same pH, there was a marked difference in urine FA concentrations between AD patients and healthy controls (Supplementary Figure 3(b) to (d)). These data confirm that FA concentrations in urine from AD patients are higher than healthy controls, which is consistent with previous results detected by HPLC<sup>13,21</sup> or other methods.<sup>29</sup> Third, we observed that a very few pH values of urine samples were lower 6.0; therefore, the effects of the same sample at different pH on urine FA concentrations need to be investigated. We found that there was a negative relationship between pH value and urine FA concentration (Supplementary Figure 3(e)). Moreover, FA concentration of urine sample at pH 6.2 was higher than the same sample at pH 6.8 or 7.2 (Supplementary Figure 3(f)). This result indicates that the more acidic environment can enhance the fluorescence urine FA values. This unexpected phenomenon seemingly did not support that application of this method of NaFA probe to specifically detect

urine FA. However, urine acidification from AD patients was a possible clinical biochemical character because FA can be oxidized to form formic acid, which results in urine acidification with or without enzyme-dependent pathways<sup>30</sup> (Supplementary Figure 4(a) and (b)). Thus, urine acidification of AD patients contributes to the improvement of sensitivity of this method. The pH value in the urine of a healthy human is about 6.5. We found that the slight acidifications ranging from 6.2 to 7.2 did not affect the detected FA concentrations in urine of healthy controls (Supplementary Figure 3(f)). However, if by any chance low pH of urine from healthy controls leads to an abnormally high concentration of FA, the doctors can easily distinguish between AD and healthy controls according to the clinical scores of MMSE by cognitive examination. Therefore, this Na-FA probe can sensitively and specifically detect urine FA of AD patients.

## Conclusion

Using the NaFA fluorescent probe, an abnormally high concentration of urine FA was observed in AD patients than age-matched controls. The present fluorescent spectrophotometric approach is a simple, rapid, sensitive and practical alternative for diagnosing dementia or monitoring medicine metabolism.

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## Contributorship

All authors declare that they have intellectually participated in this work and are solely responsible for its results.

## Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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## Ethical approval

This clinical investigation (2014SY39) was approved by the Ethics Committee at the Capital Medical University, China.

## Guarantor

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