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Sensitive determination of endogenous hexanal and heptanal in urine by hollow-fiber liquid-phase microextraction prior to capillary electrophoresis with amperometric detection

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ABSTRACT

Hexanal (Hex) and heptanal (Hep) in human blood have been regarded as potential biomarkers of lung cancer. In this work, a hollow-fiber liquid-phase microextraction (HF-LPME) method has been developed for the preconcentration of these trace aldehydes in urine samples. After derivatization with an electroactive compound 2-thiobarbituric acid, these two non-electroactive aldehydes were converted to electroactive adducts, therefore detectable by capillary zone electrophoresis with amperometric detection (CZE-AD) approach. Experimental conditions of derivatization, extraction, electrophoretic separation and detection were optimized. Under the optimum conditions, the enrichment factors for Hex and Hep could reach 320 and 355, respectively. The limits of detection for Hex and Hep were 2.7 and 0.97 nM, respectively; the average recoveries were in the range of 61–95% and relative standard deviation (RSD) values less than 8.5%. The present method has been applied to quantitative analysis of two biomarkers in human urine in lieu of blood samples, and the assay results showed that the contents of Hex (0.99–6.7 μM) and Hep (2.5–6.4 μM) found in the urine sample of the lung cancer patients were significantly higher than those in the healthy volunteers, liver cancer patients, as well as diabetics. The proposed HF-LPME/CZE-AD method may provide a potential alternative for early non-invasive diagnosis of lung cancer disease.

1. Introduction

The low-molecular-weight aldehydes are a kind of important products of lipid peroxidation as a result of radical-induced oxidative stress [1], which are reactive with biological nucleophiles [2,3]. The lipid peroxidation process has been linked with various pathological conditions such as carcinogenesis [4,5], atherosclerosis [6], etc. In recent years, aldehydes in various human biological sample matrices, including in breath [7,8], blood [9–15], plasma [16–18], urine [19–27], buccal cells [28] have also been studied extensively. The presence of the aldehydes is considered a marker, and as evidence that free-radical mediated reactions have taken place. In particular, hexanal (Hex) and heptanal (Hep) in human blood have been regarded as biomarkers of lung cancer [29,30].

Nevertheless, direct determination of aldehydes is very difficult due to their properties of high volatility and activity [12]. In practice, derivatization has played an important role to overcome these problems. Generally, 2,4-dinitrophenylhydrazine (2,4-DNPH) [13–17,20–23] and o-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine hydrochloride (PFBHA) [9,10,28] are used as the derivatization reagents; and the formed adducts are separated by chromatographic techniques coupled with sensitive detectors [31]. Of which, 2,4-DNPH is the most widely used derivatization agent. However, Uchiyama and co-workers found this derivatization method was subject to analytical errors because DNP-hydrazone adducts have both E- and Z-geometrical isomers [32]. In our previous studies, an electroactive compound 2-thiobarbituric acid (TBA) was selected as an ideal reagent for derivatization of some low-molecular-weight aldehydes including formaldehyde, acetaldehyde, propanal, butanal, pentanal, hexanal, glutaradehyde, 2,3-butanedione and methylgloxal [29,33,34]; and capillary electrophoresis with amperometric detection (CE-AD) offers many

Abbreviations: CE, capillary electrophoresis; AD, amperometric detection; EF, enrichment factor; HF-LPME, hollow-fiber liquid-phase microextraction; LODs, limits of detection; Hep, heptanal; Hex, hexanal; RSD, Relative standard deviation; SCE, saturated calomel electrode

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desirable features for portable/disposable CE systems because of high sensitivity, tunable selectivity and compatibility with advanced micromachining technology [35–37].

However, the experimental results indicated that the efficiency of the derivatization reaction decreased with increasing carbon chain of low-molecular-weight aliphatic aldehydes [33]. Furthermore, owing to the complexity of sample matrices and the low level of aldehydes in the biological samples, sample cleanup and enrichment procedure are necessary to improve the sensitivity of the method. Solid phase microextraction (SPME) is a commonly used method for the extraction of aldehydes in complex samples [10,12,19,21–23,27,30], and its main advantages are solvent-free, simple and rapid. However, these methods based on SPME still have the following shortcomings: (1) SPME fiber is relatively expensive; (2) the SPME polymer coating is fragile and easily broken; and (3) sample carryover is sometimes difficult or impossible to be eliminated [9]. Therefore, it is desirable to develop a new, solvent-free, and inexpensive enrichment technique for the analysis of the biomarkers in biological samples. Recently, several liquid-phase microextraction (LPME) methods such as single-drop microextraction (SDME) [9,13,28], dispersive liquid–liquid microextraction (DLLME) [14,15] have been used for the extraction of organic compounds from environmental and biological samples. Hollow-fiber liquid-phase microextraction (HF-LPME), introduced by Pedersen-Bjergaard and Rasmussen [38], has been gained popular attention in the field of sample preparation because of its good sample purification and interference avoiding abilities as well as low organic solvent consumption, which is in accordance with the current trends to a 'Green Chemistry'. It overcomes several disadvantages to a certain extent resulted from above microextraction methods.

In the present work, based on HF-LPME, capillary zone electrophoresis (CZE), AD, and TBA derivatization, a novel hyphenated technique of HF-LPME/CZE-AD has developed for the first time to sensitively determine two long chains of aliphatic aldehydes, Hex and Hep, in non-invasive urine samples. As an electroactive species, TBA can make aldehyde adducts also becoming electroactive after derivatization, thus to facilitate the determination of Hex and Hep based on CZE-AD. The parameters involved in electrophoretic separation, AD and enrichment factors (EFs) of two biomarkers were optimized, and the proposed method has been applied to analyze real-world urine samples from healthy volunteers, lung cancer, liver cancer and diabetes patients. The detailed investigation was reported herein.

2. Material and methods

2.1. Reagents and materials

The standard compounds of Hex ($>99.9\%$) and Hep (97%) were purchased from Sigma-Aldrich (St. Louis, MO, USA), TBA ($\geq 98.5\%$) was purchased from China National Pharmaceutical Group Corporation (Shanghai, China), and they were all used as received. All other chemicals were of analytical grade. The stock solutions of Hex (0.083 M) and Hep (0.074 M) were prepared with ultra-pure water (ultrapure water meter, Shanghai Taihe Instrument Co., Ltd.). TBA (0.05 M) was prepared in ethanol solution (1:1), and all standard solutions were newly prepared every 3 days. Before use, all solutions were filtered through 0.22 μm nylon filters. The standard stock solutions and working solution were stored at 4 °C.

2.2. Apparatus

The laboratory-built CE-AD system was employed and described previously [29,33]. This system was mainly composed of a 30 kV high-voltage dc-power supply (Shanghai Institute of Nuclear Research, China), fused silica capillary (25 mm i.d., 360 mm o.d., Polymicro Technologies, Phoenix, AZ, USA), a three-electrode cell system, and a BAS LC-3D amperometric detector (Bioanalytical Systems, West Lafayette, IN). The three-electrode cell system consists of a 300-mm diameter carbon disk working electrode, a platinum auxiliary electrode, and a saturated calomel electrode (SCE) as the reference electrode. In order to avoid contact between the operator and the high voltage and assure the safety of the CE-AD system, the buffer reservoirs for CE, the entire capillary and all electrodes were enclosed in a Plexiglas box. The fused-silica capillary needed online rinse with running buffer for 30 min between electrophoretic runs. Data acquisition and analysis were performed using HW-2000 software package, version 2.21 (Shanghai Qianpu Software Co., Ltd., Shanghai, China).

2.3. Preparation of the hollow fiber

Q3/2 Accurel PP polypropylene microporous hollow-fiber membrane (200 μm wall thickness, 600 μm inner diameter, 0.2 μm pore size, and 75% porosity) were obtained from Membrana (Wuppertal, Germany). The hollow fiber was cut into segments with a length of 5 cm, and the approximate internal volume of each segment was ~10 μL. The hollow fiber units were sonicated for 1 min in HPLC-grade acetone to remove any contaminants in the fiber. After sonication, the fibers were removed from the acetone, and the solvent was allowed to evaporate completely. A hollow-fiber segment was subsequently placed between the two needle ends and dipped for a 30 s period into 1-octanol as the organic solvent. After impregnation, air was flushed through the hollow fiber with a 10 μL microsyringe (with a needle of 0.3 mm outer diameter and 5.5 cm length, Shanghai Guangzheng Medical Instruments Co., Ltd., Shanghai, China) to remove excess organic solvent from the inside of the fiber.

And then, the one end of the hollow fiber was sealed, 10 μL of acceptor solution (0.3 M NaOH) was introduced carefully without penetration into the wall of the hollow fiber; otherwise, it would be lost during the extraction. Then, the other end of the hollow-fiber segment was heat-sealed.

2.4. Sample preparation, derivatization and extraction procedure

Urine samples of healthy volunteers were collected randomly from students in our laboratory, and those of patients with lung cancer, liver cancer or diabetes were collected in Huashan Hospital (Shanghai, China) and People's Hospital of Shanghai Putuo District (Shanghai, China), respectively. All samples were stored at −20 °C. Before use, each thawed urine sample (about 3 mL) was centrifuged for 10 min (speed of 10,000 rpm), and then filtered through 0.22 μm syringe filter. 2 mL of filtrate was derivatized by adding 750 μL TBA (0.05 M) as a derivatization reagent, 750 μL hydrochloric acid (1.45 M) and some ultra-pure water to a total volume of 4 mL in a 5 mL flask. The flask was capped and shaken slowly under the effect of magnetic stir, and the reaction proceeded for 60 min at 35 °C. The optimization of extraction procedure was conducted using a unified standard solution (Hex, 0.83 μM; Hep, 0.74 μM). The detailed extraction and preconcentration procedure for target analytes in urine samples was according to the following procedure. An 8 mL of mixed standard solution (pH adjusted with 0.6 M HCl to the final concentration of 0.3 M (pH=0.52)) was placed in a 10 mL beaker, and a small stirring bar was placed in the solution to ensure efficient stirring during the extraction, which was covered with aluminum foil to prevent evaporation. Then, the two-end sealed hollow fiber, filled with the acceptor phase, was placed in the center of this spot for extraction of the analytes. At the same time, the magnetic stirrer (100–2000 rpm) was switched

on to start the dynamic extraction at 400 rpm. After a prescribed time, the magnetic stirrer was switched off and the hollow fiber was removed from the sample solution. One end of the hollow fiber was cut carefully with a sharp blade and the needle tip of the microsyringe was carefully inserted into the hollow fiber. The acceptor solution in the hollow fiber (0.3 M NaOH, pH=13.48) was withdrawn into the syringe, and was ready for the CZE-AD analysis. Each piece of hollow fiber was used only for a single extraction.

2.5. Method validation

To determine the linearity between the detection signal (peak area) and concentration of Hex–TBA and Hep–TBA adducts, a series of standard solutions containing different concentrations (Hex, 2.00–200 μM; Hep, 1.75–175 μM) were tested. The peak area and concentration of analytes were subjected to regression analysis to calculate the calibration equation and correlation coefficient. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated on the basis of $S/N=3$ and $S/N=10$, respectively. The reproducibility of the method was evaluated by intraday precision and interday precision. The relative standard deviation (RSD) was used as a measure of precision. The intraday precision was assessed by making seven repetitive injections of the mixed standard solution of two biomarkers under the selected optimum conditions at three different concentrations (Hex, 83, 40, and 8.0 μM; Hep, 74, 35, and 7.0 μM). The interday precision was estimated by making repetitive injections of a mixed standard solution (Hex, 83 μM; Hep, 74 μM) for five consecutive days for three replicates. To further evaluate the reliability of the method, recovery experiments were performed by a standard addition method. Under the optimum conditions, recovery data were determined with the real urine samples of no.1 healthy volunteer and no.1 lung cancer patient at three concentration levels (Hex, 80, 40, and 8.0 μM; Hep, 70, 35, and 7.0 μM), respectively.

3. Results and discussion

3.1. Optimization of CZE-AD analytical conditions

Since the aldehyde–TBA adducts can be readily oxidized electrochemically at a relatively moderate potential, AD was used in this work. The hydrodynamic voltammetries (HDVs) of two biomarkers were investigated to obtain optimum detection results (as shown in Fig. 1 of Supplementary material). When the applied potential exceeded +0.40 V (vs. saturated calomel electrode, SCE), two biomarkers could generate oxidation current at the working electrode, and the peak area of Hex–TBA adduct increased rapidly. When the applied potential was greater than +0.95 V (vs. SCE), although the peak areas of two biomarkers were still increased, the baseline noise and the background current increased very strongly, resulting in unstable baseline, which is a disadvantage for sensitive and stable detection. Therefore, the applied potential to the working electrode was maintained at +0.95 V (vs. SCE), and the working electrode showed good stability and high reproducibility at this optimum potential.

The running buffer pH value also plays an important role in CE because it effects on zeta potential, EOF, as well as the overall charge of all analytes, which together affect the migration time and the separation of the analytes. Therefore, the effect of the running buffer pH on the migration time of the analytes was investigated in the pH range of 9.35–11.04 (as shown in Fig. 2 of Supplementary material). The experimental results showed that the resolution of two biomarkers was improved with the increasing pH value; and four adjacent aliphatic aldehyde homologs, including pentanal, Hex, Hep, and octanal, could achieve baseline separation when the pH value was greater than 9.66.Therefore, in considering the analytical time and stability, pH 9.66 was selected as the optimum pH value for further investigation.

Besides, other factors including buffer concentration, separation voltage and injection time were also investigated individually. Under the optimum conditions, two biomarkers could be well separated from their aldehyde homologs as well as the main co-existing electrochemical interference compounds in urine samples in 80 mM $Na_2B_4O_7$–NaOH buffer (pH 9.66) at the separation voltage of 12 kV within 32 min; the applied potential to the working electrode was +0.95 V (vs. SCE), and the injection time was 10 s (at 12 kV). (The electropherogram of a mixed standard solution of four adjacent aliphatic aldehyde homologs was shown in Fig. 3 of Supplementary material.)

3.2. Optimization of derivatization reaction

The temperature is a fundamental parameter for derivatization. Generally, increasing the temperature will accelerate the reaction. In order to study the effect of temperature on the reaction yield, a series of mixed standard solutions of Hex (0.83 mM) and Hep (0.74 mM) were incubated with TBA (9.4 mM) at 25 °C, 35 °C, 45 °C, 55 °C and 65 °C, respectively. As shown in Fig. 1A, the peak areas of Hex–TBA and Hep–TBA adducts increased with the increasing of the temperature at the start stage; while their peak areas became decreased with the elevated temperature when the temperature was higher than 35 °C. A high temperature may cause the side-reaction increase of two aldehydes or the decomposition of adducts. Therefore, the value of 35 °C was selected for further studies.

Reaction time is another important parameter, which in a great extent affects the detection sensitivity of the method. The effect of the reaction time on the adduct yields was investigated in the range of 0–90 min (at 35 °C) as shown in Fig. 1B. It was found that the yield of two adducts increased quickly when the reaction time was less than 60 min, while the yield was decreased slowly after 60 min. Therefore, the reaction time of 60 min was chosen as the optimal derivatization time because it could provide satisfactory detection sensitivity and adequate sample throughput.

The effect of TBA amount on the derivatization was investigated to obtain the optimum TBA concentration. Accurate amount of Hex (0.83 mM) and Hep (0.74 mM) were transferred into a flask and then different concentration of TBA (3.1–15.7 mM) solution was added so that the two aldehydes could be fully derivatized. The experimental results showed that no significant increase of peak area of each adduct was observed when the mole ratio of aldehydes vs. TBA (mol/mol) was above 1:6 (as shown in Fig. 4 of Supplementary Material). Besides, a high reagent ratio will push the reaction to the side of product formation. Therefore, the optimum ratio of each aldehyde vs. TBA was 1:6. Once formed, these adducts were highly stable and showed no significant change in average peak area up to 12 h.

3.3. Optimization of enrichment conditions for HF-LPME

To obtain the optimal extraction efficiency, various parameters that potentially affect sample extraction were studied. Optimization of the procedure was based on the one-factor-at-a-time approach, in which the effect of one parameter is investigated and all other parameters are kept constant.

In three-phase HF-LPME, the type of organic solvent plays an important role in the extraction efficiency and analyte preconcentration. Therefore, several requirements should be considered in order to achieve satisfactory enrichment efficiency. The commonly-used organic solvents such as 1-octanol, toluene and ether have

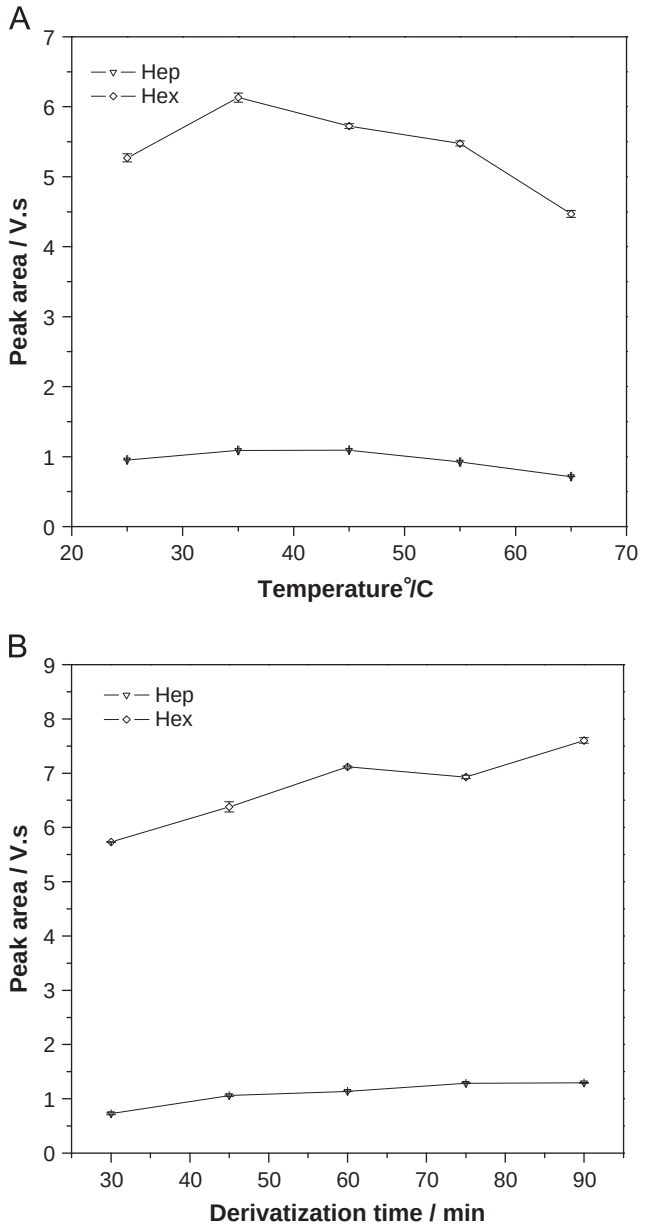

Fig. 1. Effects of derivatization temperature (A) and derivatization time (B) on the peak areas of two biomarkers. Optimum derivatization conditions: (1) derivatization temperature, 35 °C; (2) derivatization time: 60 min; and (3) derivatization ratio, 1:6. The concentrations of analytes were 83 μM (Hex–TBA) and 74 μM (Hep–TBA), respectively.

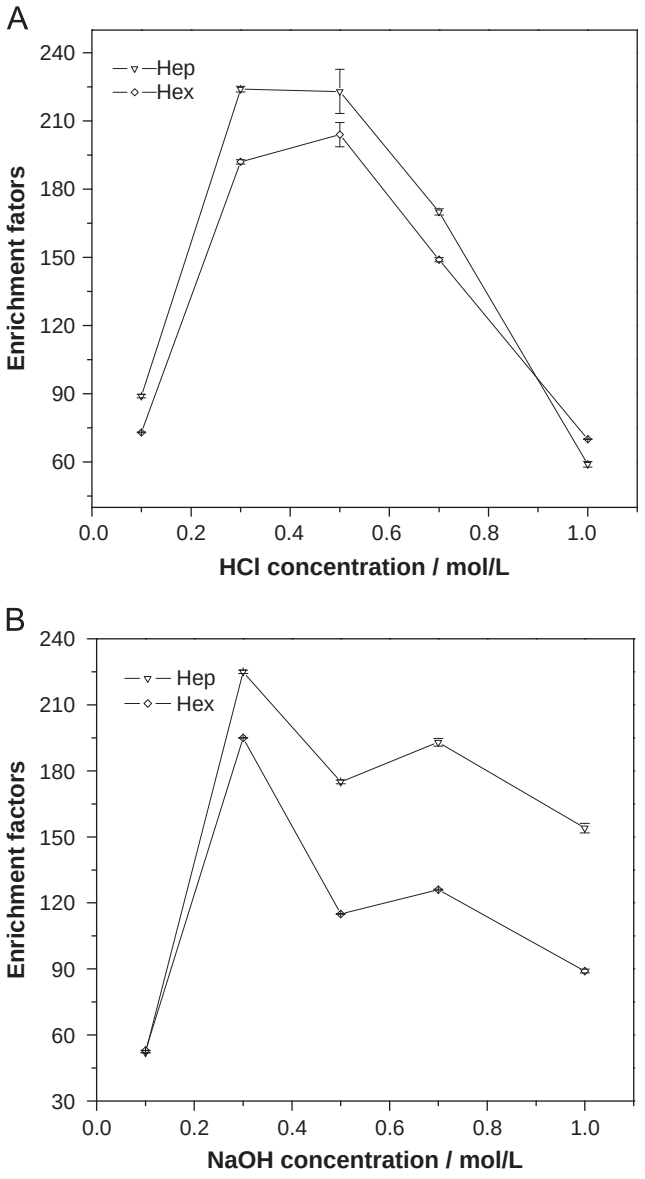

Fig. 2. Effects of the acidity of donor phase (A) and the basicity of acceptor phase (B) on EFs of two biomarkers. Optimum extraction conditions: (1) extraction organic solvent, 1-octanol; (2) donor phase, 0.3 M HCl; (3) acceptor phase, 0.3 M NaOH; and (4) extraction tine, 75 min. The concentrations of analytes were 83 μM (Hex–TBA) and 74 μM (Hep–TBA), respectively.

been examined in this work. The experimental results showed that the liquid membrane of toluene and ether were unstable, resulting in the loss of the acceptor solution; while 1-octanol offered better enrichment characteristics for the target analytes and exhibited good reproducibility. Therefore, 1-octanol was selected as the extraction solvent for further studies.

The compositions of both donor and acceptor phases are very important parameters which affect the extraction efficiency in HF-LPME. The pH of the donor phase is adjusted to deionize the analytes, while the acceptor phase is adjusted to ionize them. The difference in pH value between the donor and acceptor phases is one of the major parameters which can promote the transfer of analytes from donor to acceptor phase. Hence, a series experiments were conducted to obtain the optimum compositions of the donor and acceptor phases. As shown in Fig. 2A, the HCl concentration was studied in the range of 0.1–1.0 M (pH: 1–0). At first a significant enhancement of the extraction efficiencies could be obtained with the increasing of HCl concentration (0.1–0.3 M); and the enrichment factors (EFs) of two biomarkers were leveled off in the range of 0.3–0.5 M; however, EFs of two biomarkers had a sharp decline when the concentration was over than 0.5 M, therefore, 0.3 M HCl (pH=0.52) was used as donor media taking into account the urine sample itself has a certain acidity. In addition, the NaOH concentration was also studied in the range of 0.1–1.0 M (pH: 13–14) as shown in Fig. 2B, and there was an obvious inflection point of the EF values at the concentration of 0.3 M. Therefore, 0.3 M NaOH (pH=13.48) was selected as acceptor media which could provide relative high EFs for the target analytes.

The effect of extraction time on EFs of the target analytes has also been investigated in the range of 0–90 min (as shown in Fig. 5 of Supplementary Material). The assay results showed that the EFs of two biomarkers increased quickly with extraction time up to 75 min; and then the EFs began to decrease with the

extension of extraction time. Since the extraction is an equilibrium process, and a proper extraction time is necessary to obtain high extraction efficiency; however, the possible redissolution of the analytes in the donor phase could result in the diminution of the EFs at longer extraction time. In order to balance the extraction efficiency and analytical time, 75 min was chosen as the optimum time in this work.

The effects of common-used salts including NaCl, KCl and Na_2SO_4 have been also investigated by adding various amounts of salt (0–2.5 M) into the sample solution, respectively. The results showed that the EFs of two biomarkers had no significant increase when the NaCl concentration was lower than 1.7 M; while the EFs of the target analytes decreased remarkably when the salt concentration was higher than 1.7 M. And, the experiments of KCl and Na_2SO_4 obtained the similar results. So, the above salts were not used in this experiment.

Table 1
The regression equations and LODs of two biomarkers ($n=3$)[a].

Biomarkers	Regression equation[b]		r	Linear range (μM)	EFs	LOD (nM)	LOQ (nM)
	Slope (RSD%)	Intercept (RSD%)					
Hex	0.090 (0.16)	−0.053 (3.2)	0.9996	4.0–100	320	2.7	9.0
Hep	0.020 (0.71)	−0.004 (0.0)	0.9995	3.5–88	355	0.97	3.2

[a] HF-LPME/CZE-AD conditions were the same as those in Figs. 2 and 3, respectively.
[b] In the regression equation, the unit of analyte concentration was μM, and that of the peak area was V. s.

3.4. Method validation

As shown in Table 1, an excellent linearity ($r \geq 0.9995$) between peak area and analyte concentration could be obtained at three orders of magnitude. The LODs and LOQs of two biomarkers were 2.7 nM, 9.0 nM for Hex–TBA adduct and 0.97 nM, 3.2 nM for Hep–TBA adduct, respectively. The two aldehyde–TBA adducts could be pre-concentrated up to 355-fold.

The experiment for intraday precision showed that the RSDs of peak area and migration time were within 3.4%, 2.2% for Hex–TBA adduct, 4.0%, 2.2% for Hep–TBA adduct, respectively. And that for interday precision revealed that the RSDs of peak areas and migration time were within 4.6% and 2.1%, respectively. The repeatability data indicated that it was feasible to determine the aldehyde biomarkers based on the proposed CZE-AD method.

The recovery data were shown in Table 1 of Supplementary Material, and the average recovery was in the range of 61–95% with corresponding RSDs of 2.2–8.5%, which indicated that the HF-LPME/CZE-AD method was sufficiently accurate for the simultaneous determination of the target compounds.

Table 2
The assay results of two biomarkers in human urine samples ($n=3$).[a]

Subjects	Hex (μM)	Hep (μM)
Healthy volunteers ($n=15$)	0.12–0.97	0.09–1.3
Lung cancer patients ($n=10$)	0.99–6.7	2.5–6.4
Liver cancer patients ($n=5$)	0.30–0.67	0.26–0.84
Diabetics ($n=5$)	0.13–0.80	0.26–0.74

[a] HF-LPME/CZE-AD conditions were the same as those in Figs. 2 and 3.

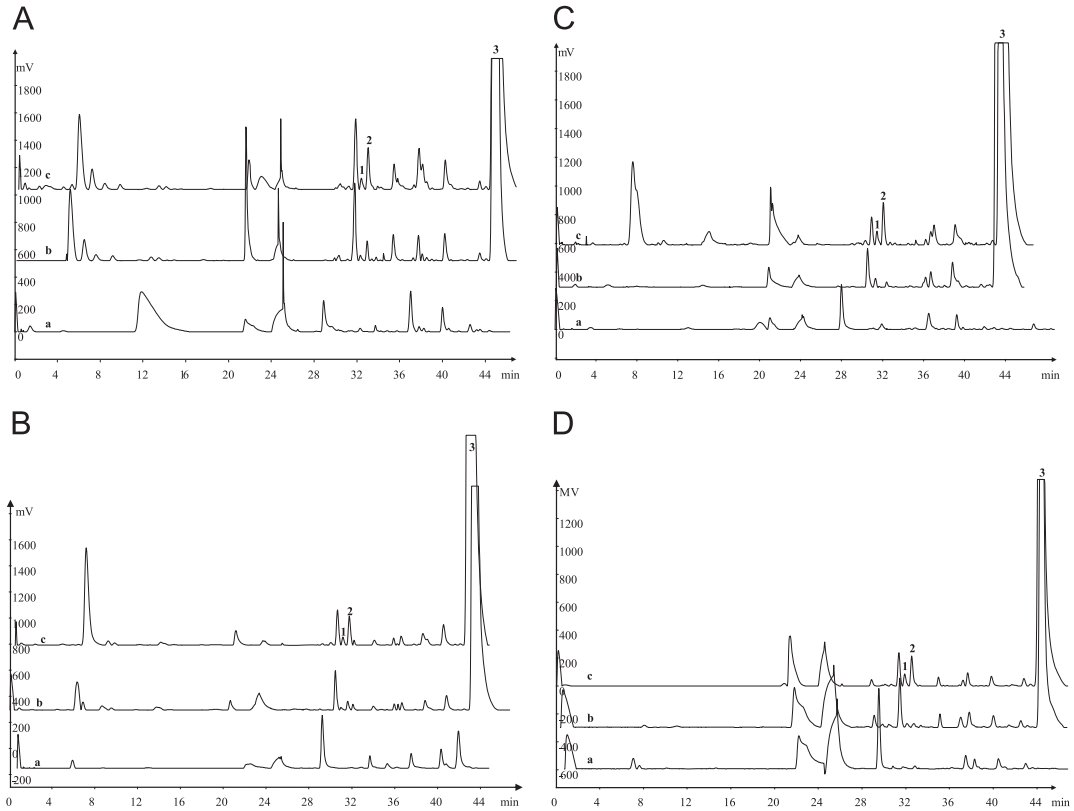

Fig. 3. Electropherograms of (a) blank without TBA derivatization, (b) blank with TBA derivatization, and (c) spiked urine samples (Hex–TBA, 80 nM; Hep–TBA, 70 nM) from lung cancer patient (A), liver cancer patient (B), diabetics (C) and healthy volunteer (D), respectively. Fused-silica capillary: 25 μm i.d. × 90 cm; working electrode: 300 μm diameter carbon-disc electrode; working electrode potential: +0.95 V (vs. SCE); running buffer: 80 mM $Na_2B_4O_7$–NaOH buffer (pH 9.66); separation voltage: 12 kV; injection time: 10 s (at 12 kV); peak identifications: (1) Hep–TBA; (2) Hex–TBA, and (3) TBA.

Table 3
Comparison of this method with the reported methods for determination of Hex and Hep in urine and blood samples.

Sample	Method	EFs	LODs (nM)	Recovery (%)	Ref.
Blood	DLLME/HPLC-UV		2.34–7.90	60.8–70.2	[14]
	DLLME/HPLC-UV		2.4–3.6	83–87	[17]
	DLLME/LC–APCI-MS–MS	63–73	0.0176–0.71		[15]
Urine	CE-MS	123	150–180	44.7–54.5	[20]
	HS-SPME-GC–MS		0.88–1.10	66.3–70.5	[19]
	MSPE-HPLC-UV		0.78–1.87	82.5–99.4	[21]
	SPME-HPLC-UV		0.76–0.81	70.71–80.83	[22]
	HS-SPME-GC–MS		0.11 (Hex)		[27]
	SDME/GC–MS		0.12–0.16	86–90	[9]
	PME-GC–MS		0.005–0.006	89–95	[10]
	SPME-GC–MS		0.026–0.032	96–98	[30]
	HF-LPME/CZE-AD	320–355	0.97–2.7	61–95	In this work

3.5. Applications

Under the optimum conditions, the proposed method was applied for determining Hex and Hep in real urine samples by the developed HF-LPME/CZE-AD method. The typical sample electropherograms were shown in Fig. 3A–D, respectively; and three electropherograms (as shown in a–c) for each type of urine sample represented blank sample without TBA derivatization, blank sample with TBA derivatization, and spiked urine samples (Hex, 80 nM; Hep, 70 nM), respectively. By a standard addition method and comparing the migration time of target analytes with those of the mixed standard solution electropherogram, Hex and Hep were determined in all urine samples. From the sample electropherograms, we can see that the two biomarkers could be well separated from the main coexisting substances in the real-world urine samples as well as TBA under the optimized experimental conditions.

The detailed data for the tested samples were summarized in Table 2. The assay results showed that the contents of Hex and Hep found in the urine samples of lung cancer patients were in the range of 0.99–6.7 μM and 2.5–6.4 μM, respectively, while, those in healthy volunteers were 0.12–0.97 μM and 0.090–1.3 μM. It is obvious that the contents of two aldehydes for lung cancer patients were higher than those for healthy volunteers. Besides, there were no obvious content differences of the two aldehydes for diabetics and liver cancer patients comparing with those of healthy volunteers. The assay results were approximately equal to the reported values in literatures [20,22], indicating that Hex and Hep in human urine can be recommended as biomarkers of lung cancer in early non-invasive diagnosis.

4. Conclusions

The above experimental results demonstrated the capability of HF-LPME/CZE-AD method for sensitive determination of two long chains of aliphatic aldehyde biomarkers, Hex and Hep, in urine samples. As shown in Table 3, the LODs of two aldehyde biomarkers obtained by the proposed method were nearly equivalent and even better than those of most of reported methods except SPME/GC–MS approach [10,32]; and relatively good recoveries (61–95%) were obtained for the real-world urine samples. Furthermore, high extraction efficiency as well as low analytical cost validated the feasibility of the proposed method in the analysis of complex biological samples. The proposed HF-LPME/CZE-AD method may provide an alternative for early non-invasive diagnosis of lung cancer disease. In order to collect statistically significant biological data, further investigations were suggested to discriminate between the pathological state of lung cancer patients and physiological conditions of healthy subjects, using the simple, rapid and economic method here reported for the quantification of urinary aldehydes.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.10.052.

References

[1] B. Halliwell, Lipid Peroxidation, Free-Radical Reactions and Human Disease, Current Concepts, The Upjohn Co., Kalamazoo, MI, 1991.
[2] H.D. Hoberman, R.C.S. George, J. Biochem. Toxicol 3 (1988) 105–119.
[3] V.L. Wilson, P.G. Foiles, F.L. Chung, A.C. Povey, A.A. Frank, C.C. Harris, Carcinogenesis 12 (1991) 1483–1490.
[4] A.K. Basu, L.J. Marnett, Carcinogenesis 4 (1983) 4331–4333.
[5] N. Bekerecioglu, R. Asla, S. Ugras, A. Kutluhan, R. Sekeroglu, N. Akpolat, M. Karakok, Eur. J. Plast. Surg. 21 (1998) 227–229.
[6] R. Chiesa, G. Melissano, R. Castellano, D. Astore, E.M. Marone, A. Grossi, E. Maggi, G. Finardi, A. Casasco, G. Bellomo, Ann. Vasc. Surg. 12 (1998) 1–9.
[7] P. Fuchs, C. Loeseken, J.K. Schubert, W. Miekisch, Int. J. Cancer 126 (2010) 2663–2670.
[8] X. Chen, M.F. Cao, Y. Li, W.J. Hu, P. Wang, K.J. Ying, H.M. Pan, Meas. Sci. Technol. 16 (2005) 1535–1546.
[9] N. Li, C.H. Deng, N. Yao, X.Z. Shen, X.M. Zhang, Anal. Chim. Acta 540 (2005) 317–323.
[10] C.H. Deng, N. Li, X.M. Zhang, J. Chromatogr. B 813 (2004) 47–52.
[11] I. Rasanen, J. Viinamaki, E. Vuori, I.J. Ojanpera, Anal. Toxicol. 34 (2010) 113–121.
[12] R.Y. Xue, L. Dong, S. Zhang, C.H. Deng, T.T. Liu, J.Y. Wang, X.Z. Shen, Rapid Commun. Mass Spectrom. 22 (2008) 1181–1186.
[13] H. Xu, L.L. Lv, S. Hu, D.D. Song, J. Chromatogr. A 1217 (2010) 2371–2375.
[14] L.L. Lv, H. Xu, D.D. Song, Y.F. Cui, S. Hu, G.B. Zhang, J. Chromatogr. A 1217 (2010) 2365–2370.
[15] H. Xu, D.D. Song, Y.F. Cui, S. Hu, Q.W. Yu, Y.Q. Feng, Chromatographia 70 (2009) 775–781.
[16] H. Xu, Z.H. Yan, D.D. Song, J. Sep. Sci. 35 (2012) 713–720.
[17] H.J. Zhang, J.F. Huang, B. Lin, Y.Q. Feng, J. Chromatogr. A 1160 (2007) 114–119.
[18] M. Matsuoka, N. Imado, T. Maki, K. Banno, T. Sato, Chromatographia 43 (1996) 501–506.
[19] R. Guadagni, N. Miraglia, A. Simonelli, A. Silvestre, M. Lamberti, D. Feola, A. Acampora, N. Sannolo, Anal. Chim. Acta 701 (2011) 29–36.
[20] H. Xu, Z.H. Yan, X.X. Guo, J. Huazhong Norm. Univ. (Nat. Sci.) 46 (2012) 49–54.
[21] D.D. Song, Y.Q. Gu, L. Liang, Z.H. Ai, L.Z. Zhang, H. Xu, Anal. Methods 3 (2011) 1418–1423.

[22] H. Xu, S.Y. Wang, G.B Zhang, S.Q. Huang, D.D. Song, Y.P. Zhou, G.D. Long, Anal. Chim. Acta 690 (2011) 86–93.
[23] C.E. Banos, M. Silva, J. Chromatogr. B 878 (2010) 653–658.
[24] M. Eggink, M. Wijtmans, R. Ekkebus, H. Lingeman, I.J.P. de Esch, J. Kool, W.M.A. Niessen, H. Irth, Anal. Chem. 80 (2008) 9042–9051.
[25] W.J. Goux, G. Luta, Anal. Chem.: Indian J. 2 (2006) 81–91.
[26] S.S. Kim, D.D. Gallaher, A.S. Csallany, Lipids 34 (1999) 489–496.
[27] M. Kusano, E. Mendez, K.G. Furton, Anal. Bioanal. Chem. 400 (2011) 1817–1826.
[28] N. Li, C.H. Deng, X.Y. Yin, N. Yao, X.Z. Shen, X.M. Zhang, Anal. Biochem. 342 (2005) 318–326.
[29] J.B. Zhang, H.T. Zhang, M.J. Li, D.L. Zhang, Q.C. Chu, J.N. Ye, J. Chromatogr. A 1217 (2010) 5124–5129.
[30] C.H. Deng, X.M. Zhang, N. Li, J. Chromatogr. B 808 (2004) 269–277.
[31] A.K.K.V. Pillai, K. Gautam, A. Jain, K.K. Verma, Anal. Chim. Acta 632 (2009) 208–215.
[32] S. Uchiyama, Y. Inaba, M. Matsumoto, G. Suzuki, Anal. Chem. 81 (2009) 485–489.
[33] J.B. Zhang, M.J. Li, W.L. Li, Q.C. Chu, J.N. Ye, Electrophoresis 32 (2011) 705–711.
[34] D.L. Zhang, J.B. Zhang, M.J. Li, W.L. Li, B.H.E. Guli, B.S.T. Guli, J.N. Ye, Q.C. Chu, Food Chem. 129 (2011) 206–212.
[35] R.S. Martin, in: C.S. Henry (Ed.), Microchip Capillary Electrophoresis Methods and Protocols, Humana Press, Totowa, NJ, 2006.
[36] N. Dossi, S. Susmel, R. Toniolo, A. Pizzariello, G. Bontempelli, J. Chromatogr. A 1207 (2008) 169–174.
[37] N. Dossi, S. Susmel, R. Toniolo, A. Pizzariello, G. Bontempelli, Electrophoresis 30 (2009) 3465–3471.
[38] S. Pedersen-Bjergaard, K.E. Rasmussen, Anal. Chem. 71 (1999) 2650–2656.