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Analysis of Nucleosides and Nucleobases by Microemulsion Electrokinetic Capillary Chromatography Coupled with Field-amplified Sample Injection

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A microemulsion electrokinetic capillary chromatography (MEEKC) method was on-line coupled with field-amplified sample injection (FASI) for the analysis of nucleosides and nucleobases, namely cytidine, guanosine, N6-methyladenosine, fluorouracil, thymine, adenine, mercaptopurine, 6-hydroxypurine, guanine. A microemulsion background electrolyte containing 10 mM sodium dodecyl sulfate (SDS), 0.6% (v/v) 1-butanol, 0.5% (v/v) ethyl acetate and 98.9% (v/v) borate buffer (10 mM; pH 9.0) was used as running buffer. An on-line field-amplified sample injection (FASI) technique was adopted to improve the detection sensitivity. Baseline separation of nine nucleosides was achieved within 12 min with the detection limits (S/N=3) between 0.22 and 2.97 μg/mL with the DAD detector at 200 nm in the optimized conditions. The proposed method was applied to the determination of nine nucleoside compounds in spiked urine and serum samples with the recoveries ranged 91.2-113% and 85.2-112% and the relative standard deviation (RSDs, n=3) less than 5.90% and 8.22%, respectively.

Introduction

Nucleosides and nucleobases (i.e. nucleotides, bases and their analogues) are the primary substances constituting RNA and DNA and maintaining life activities of biological cell. Many diseases arise from the abnormality of nucleosides and their metabolites. Most of nucleosides with good physiological activities protect against herpes virus and retrovirus and have proven irreplaceability for physiology and pharmacology.[1-3] Nucleosides are playing increasing important role in antiviral, anti-tumour and anti-AIDS,[4-6] accounting for a substantial proportion of the antiviral drugs with the greatest potential after the sulfa drugs and antibiotics.[7] The drugs approved for anti-AIDS by the U.S. FDA, for example, are predominantly nucleoside derivatives. Additionally, the concentration changes of nucleosides in human urine can be a prognostic index of diseases.[7] Some modified nucleosides, as possible cancer biomarkers, have been shown to be abnormal amounts in urine of cancer patients and have been of interest since the 1970s.[8-10] Consequently, fast and efficient analysis of various nucleosides is an urgent and continuing topic in the field of natural pharmaceutical chemistry, pharmaceutical analysis and disease diagnosis.

Nowadays, thin layer chromatography (TLC),[11, 12] gas chromatography (GC),[13] liquid chromatography-mass spectrometry (LC-MS),[14, 15] capillary electrophoresis (CE)[10, 16, 17] and capillary electrochromatography (CEC)[18, 19] have been employed as common method for separation and determination of nucleoside and their derivatives. The TLC has disadvantages of low sensitivity and limit of analytes. GC method usually requires complicated derivatization steps to improve the volatility of the test compounds. LC-MS method has been demonstrated for the analysis of nucleosides compounds. However, expensive equipments and large volumes of organic

solvents are always necessary. Even though CEC is fast, efficient and less sample consumption, it is hindered by poor reproducibility, easy bubble formation, relatively long separation time and pH shifting.[17] In many cases, a more efficient separation and determination technique is required either to assess the levels of nucleosides or to further promote the efficacy of the nucleoside drugs in biomedical science.

CE has been believed as a simple, rapid method for the analysis of nucleoside and their derivatives. However, the close isoelectric point (*pI*s) of nucleosides and analogues make them difficult to be separated with conventional CE method.[10, 16, 17] In the recent decades, a modified technique known as microemulsion electrokinetic chromatography (MEEKC) with an oil-in-water (o/w) microemulsion as alternative pseudostationary phases (PSP) has been used to bioanalysis.[20-23] MEEKC combines chromatographic partitioning between two phases and electrokinetic migration. The separation mechanism is very similar to Micellar electrokinetic capillary chromatography (MEKC).[20,24,25] Furthermore, the microemulsion structure increases fluidity, aiding in analyte penetration and mass transfer. Meanwhile, the oil droplets in microemulsions can be positively or negatively charged depending on the surfactant to improve the separation.[26] By changing the surfactant concentration and subsequently altering the charge density of the aggregate, MEEKC is gifted with the ability to extend the elution range of the separation.[27] The features above which enables MEEKC the high efficiency separation of charged or neutral analytes covering a wide range of water solubility[26] and offers a large and flexible separation capability for various analytes.

The low sensitivity of MEEKC coupling with UV detector, as it happens with other CE modes, is due to the cell's short optical path length, the small size of capillary and the limited amount of sample injection.[24, 26, 28] Some sample concentration steps are therefore necessary for improving the detection limit. On-line enrichment technologies, such as field-amplified sample injection (FASI), large volume sample stacking (LVSS) and reversed electrode polarity stacking method was called for settling this dilemma of MEEKC.[22, 29-31]

The aim of our study presented here was to develop a fast, low-cost and sensitive FASI-MEEKC method for simultaneous detection of nine nucleosides and nucleobases including normal and modified nucleosides (structural formula shown in Fig.1). The effects of microemulsion composition and separation voltage were carefully chosen to optimize the separation. Sample diluents and injection conditions, the essential factors in FASI, were investigated in detail to improve the sensitivity. This method was validated for the determination of nucleoside compounds in urine and serum samples.

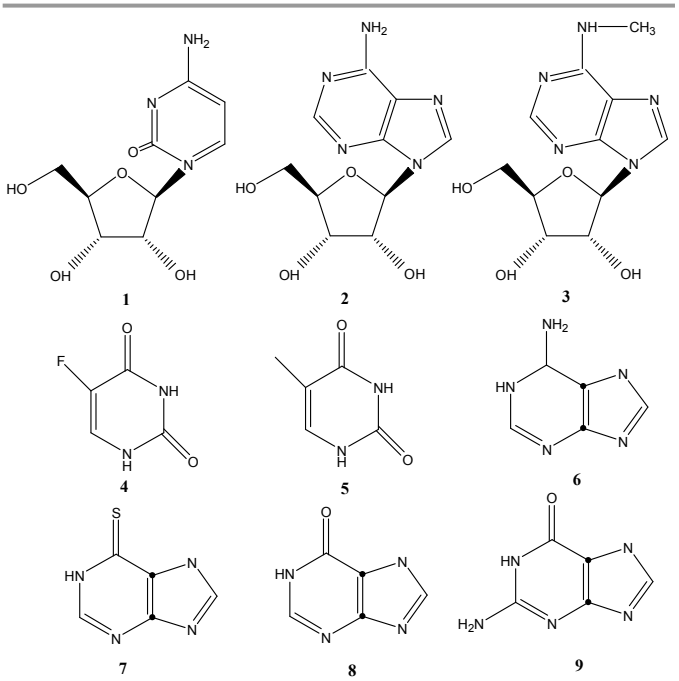

Fig.1 The chemical structures of 9 nucleoside compounds. 1, cytidine; 2, guanosine; 3, N6-methyladenosine; 4, fluorouracil; 5, Thymine; 6, Adenine; 7, mercaptopurine; 8, 6-hydroxypurine; 9, Guanine

Experimental

Chemicals

Cytidine, guanosine, N6-methyladenosine, fluorouracil, thymine, adenine, mercaptopurine, 6-hydroxypurine, guanine were obtained from J&K Chemical (Shanghai, China). Sodium dodecylsulfate (SDS) was purchased from Acros Organics (Geel, Belgium). Sodium tetraborate, 1-butanol, and ethyl acetate (analytical grade) were purchased from Kermel Chemical Reagents Development Centre (Tianjin, China). Acetonitrile (HPLC-grade) were provided by Sinopharm Chemical Reagents (Shanghai, China). An uncoated fused-silica capillary was product of Yongnian Optic Fiber Factory, (Hebei, China). Water was purified using a Milli-Q system (Millipore, Bedford, MA, USA).

Instrumentation

An Agilent CE3D system (Agilent Technologies, Waldbronn, Germany) equipped with a diode array detector (DAD) was employed for the separation and determination of the target analytes. Data acquisition and processing were performed with Agilent ChemStation software. All pH value of running buffer was measured by PHS-3C meter (Shanghai Dapu Instument Company, Shanghai, China). Prior to use, all mobile phases for MEEKC were degassed with a KQ3200E ultrasonic bath (Kunshan, China).

Preparation of running buffer for MEEKC

Borate buffer was prepared from sodium tetraborate and the pH was adjusted with sodium hydroxide or hydrogen chloride. Microemulsions were prepared by mixing the appropriate ratio of components to obtain different microemulsion. Initially, the oil was mixed with the co-surfactant, and then the buffer containing surfactant was added. The optimum microemulsion consisted of 0.5 % ethyl acetate, 0.6 % (v/v) 1-butanol and 98.9 % 10 mM borate buffer at pH 9.0 containing 10 mM SDS. The microemulsion was sonicated for 30 min to obtain the stable and optically transparent microemulsion system. The solutions were filtered through a 0.22 μm microfilter prior to use.

Preparation of standard solutions and samples

A stock standard solution of 1.0 mg/mL of each analyte was prepared in deionized water and stored at 4 ℃. The standard mixture was prepared by mixing stock standard solution and subsequently diluting with deionized water as needed. The urine and serum sample were collected from healthy male volunteer. The spiked urine samples were prepared as follow: desired amount of nine nucleoside compounds were mixed and added to urine sample. To remove the protein components and solid particles, urine samples were mixed with methanol (1:1, V/V), followed by centrifugation at 4000 rpm for 10 min, then passed through a 0.22 mm membrane filter. The collected solution was diluted 10-fold with 10 mM sodium tetraborate. The spiked serum samples were prepared in the same way. The collected solution was diluted 10-fold with 20 mM sodium tetraborate. Both spiked urine sample and serum sample were stored at -18 ℃ prior to use.

MEEKC and FASI procedures

Electrophoretic separation was carried out in a fused-silica capillary of 63 cm (54.5 cm effective length) × 50 μm i.d. × 375 μm o.d. with separation voltage of +15 kV (25 ℃). New capillary was pretreated by rinsing with water for 30 min, 0.1M NaOH for 30 min, water for 30 min, 0.1M HCl for 30 min, water for 30 min and the running buffer for 30 min. Before each running, the capillary was rinsed with 0.1 M NaOH, DI water and microemulsion electrolyte in sequence. In normal sample injection mode, sample was directly injected with a voltage +10 kV for 6 s. In FASI mode, the electrokinetic injection was performed at +22 kV for 10 s with 10 mM borate buffer (pH 9.0) used as the sample diluent.

In all experiments, the DAD wavelength was set in 200 nm for the most compromise sensitivities of all analytes.

Results and discussion

Effect of buffer

In the majority of MEEKC separations to-date, high pH buffers such as borate or phosphate with low ionic strength (5-10 mM) are preferably adopted due to a high EOF generated with a low current when voltage is applied on the capillary.[23, 26] Besides, the pH of running buffer has direct influence on ionization of analyte. In present case, the nitrogenous bases and hydroxyl groups in nucleosides imply that high pH is theoretically applied in the separation.

A series of borate buffers (10 mM) with the pH range from 8.0 to 9.5 were accordingly investigated. The other compositions of microemulsion electrolyte were initially fixed in 10 mM sodium dodecyl sulfate (SDS), 0.6% (v/v) 1-butanol and 0.5% (v/v) ethyl acetate. The result showed that the migration time and resolution increased with the increase of pH value. Considering the poor separation of N^6-methyladenosine and cytidine, mercaptopurine and fluorouracil partly as pH below 9.0, pH 9.0 was consequently selected in following experiments.

The effect of concentration (5-15 mM) was also investigated. Unsurprisingly, higher concentration led to longer migration time and higher Joule heating while better separation was obtained. The concentration of 10 mM was selected as a compromise.

Effect of surfactant

In MEEKC, the surfactant directly affects the charge and size of the microemulsion droplet, the level of ion-pairing with charged analytes and the direction and magnitude of the EOF.[32] Anionic SDS is the most commonly used surfactant in MEEKC and was employed in our study. The concentration of SDS was optimized over the range 5 to 20 mM. The higher concentration of SDS resulted in the higher charge density on the oil droplet, the lower EOF and the longer separation time. Peak tailing, poor resolution and low response sensitivity occurred as SDS concentration higher than 15 mM. Baseline separation of nucleoside compounds was obtained with short migration time while the concentration reduced to 5-10 mM. Considering the stability of the microemulsion depending on enough surfactant, a SDS concentration of 10 mM was employed for further investigation in this study.

Effect of co-surfactant

Co-surfactant molecules position themselves between the head groups of the surfactant molecules, further easing the overall ultra-low interfacial tension and electrostatic repulsion required for spontaneous microemulsion formation.[26] The chemicals typically used for these purposes include short-chain linear alcohols such as 1-butanol, which can be solubilized into the microemulsion layer to increase the mechanical strength of the composite membrane and stability of the microemulsions. It should be noted that the superfluous 1-butanol may combine with the polar groups of SDS and thereby reducing the stability of microemulsions. In the present case, the effect of the concentration of 1-butanol was investigated in the range of 0.3 - 1.2 % (v/v), the result shown in Fig.2 indicated that no apparent change of separation occurred. Accounting of the stability of

the microemulsion and separation of analytes, the final concentration of 1-butanol was set at 0.6 % (v/v).

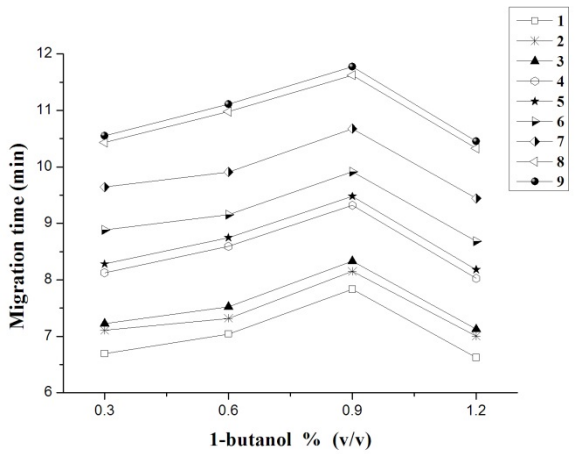

Fig.2 Effect of the concentration of cosurfactant. Conditions: 98.9 % (v/v) 10 mM borate buffer (pH 9.0) containing 10 mM SDS, 0.5 % (v/v) ethyl acetate; capillary, 63 cm (54.5 cm effective length)×50 μm *i.d.* ×375 μm *o.d.* 25℃; applied voltage, 15 kV; electrokinetic injection, +10 kV for 6 s; detection wavelength, 200 nm; temperature, 25℃. Peaks: 1, cytidine (75.0 μg/mL); 2, guanosine (50.0 μg/mL); 3, N6-methyladenosine (100 μg/mL); 4, fluorouracil (100 μg/mL); 5, thymine (75.0 μg/mL); 6, adenine (350 μg/mL); 7, mercaptopurine (350 μg/mL); 8, 6-hydroxypurine (125 μg/mL); 9, guanine (75.0 μg/mL).

Effect of oil phase

Oil, as the core phase, usually a hydrocarbon or other hydrophobic substance is enclosed by the surfactant with the aids of the co-surfactant.[24, 27, 33] Octane, ethyl acetate and cyclohexane were commonly used as the oil phase. It was concluded that under normal MEEKC conditions that variation in oil type had no significant effect on separation.[26] Ethyl acetate leads to microemulsions with a lower surface tension, meaning less surfactant was needed to stabilize the microemulsion.[30] The optimization was carried out on Ethyl acetate. The trials indicated that ethyl acetate in the range of 0.25 - 0.75 % achieved the separation of nine nucleotides without the degradation of the resolution and sensitivity. The 0.5 % (v/v) ethyl acetate was considered for the stability of microemulsion with a low concentration of SDS.

Effect of separation voltage

The magnitude and direction of EOF, the resolution and sensitivity rely on the separation voltage as well. Without doubt, the migration time of nine analytes was gradually shortened by increasing separation voltage. However, the experiments also showed that the sensitivity and resolution decreased when the separation voltage exceed +15 kV due to the Joule heating created by larger current in the running buffer. In order to obtain both good resolution and short analytical time, a separation voltage of +15 kV was applied in this study.

The optimized separation condition was eventually summarized as follows: microemulsion consist of 98.9 % (v/v) 10 mM borate buffer (pH 9.0) with 10 mM SDS, 0.6 % (v/v) 1-butanol, and 0.5 % (v/v) ethyl acetate; applied voltage, +15 kV; electrokinetic injection, 10 kV for 6 s; detection wavelength, 200 nm. Under the optimized conditions, cytidine (75.0 μg/mL); guanosine (50.0 μg/mL); N6-methyladenosine (100 μg/mL); fluorouracil (100 μg/mL); thymine (75.0 μg/mL); adenine (350 μg/mL); mercaptopurine (50.0 μg/mL); mercaptopurine (125 μg/mL); and guanine (75.0 μg/mL) were well separated and detected within 12 min (Fig.3).

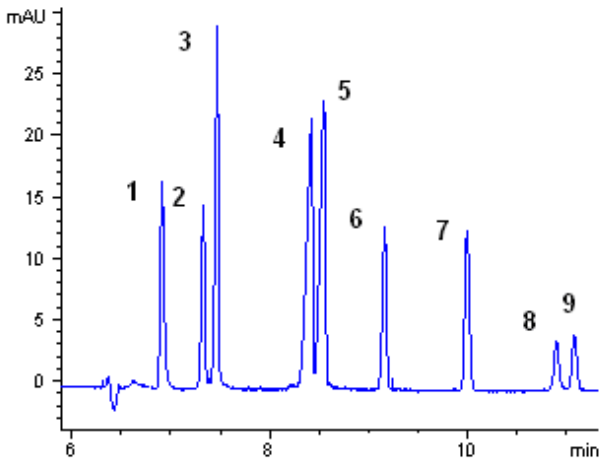

Fig.3 Electropherogram of nine nucleoside compounds in optimized MEEKC conditions. Conditions: 98.9 % (v/v) 10 mM borate buffer (pH 9.0) containing 10 mM SDS, 0.6 % (v/v) 1-butanol, and 0.5 % (v/v) ethyl acetate; applied voltage, 15 kV; electrokinetic injection, +10 kV for 6 s; detection wavelength, 200 nm. Peaks: 1, cytidine (75.0 μg/mL); 2, guanosine (50.0 μg/mL); 3, N6-methyladenosine (100 μg/mL); 4, fluorouracil (100 μg/mL); 5, thymine (75.0 μg/mL); 6, adenine (350 μg/mL); 7, mercaptopurine (350 μg/mL); 8, 6-hydroxypurine(125 μg/mL); 9, guanine (75.0 μg/mL).

Optimization of FASI

As previously stated, on-line sample pre-concentration can improve the sensitivity of MEEKC methods and make it more suitable for the biological application. In this study, FASI, stacking ionic analytes at the interface between two zones of different conductivity, was adopted to be on-line coupled with MEEKC. Before sample injection, a low conductivity solvent was introduced at the inlet of the capillary previously filled with a high ionic strength running electrolyte. Then, the sample is electrokinetically injected and analytes are concentrated at the boundary between the pre-injection. Herein, four kinds of diluents including 10 mM borate buffer (pH 9.0), 0.1 mM NaOH, methanol and the microemulsion were compared (Fig.4). We found that diluents have significant effect on both the resolution and sensitivity except 10 mM borate buffer (pH 9.0), which improve the sensitivity of all analytes with no obvious change in retention time. Consequently, 10 mM borate buffer (pH 9.0) was selected as the diluents of sample for farther research.

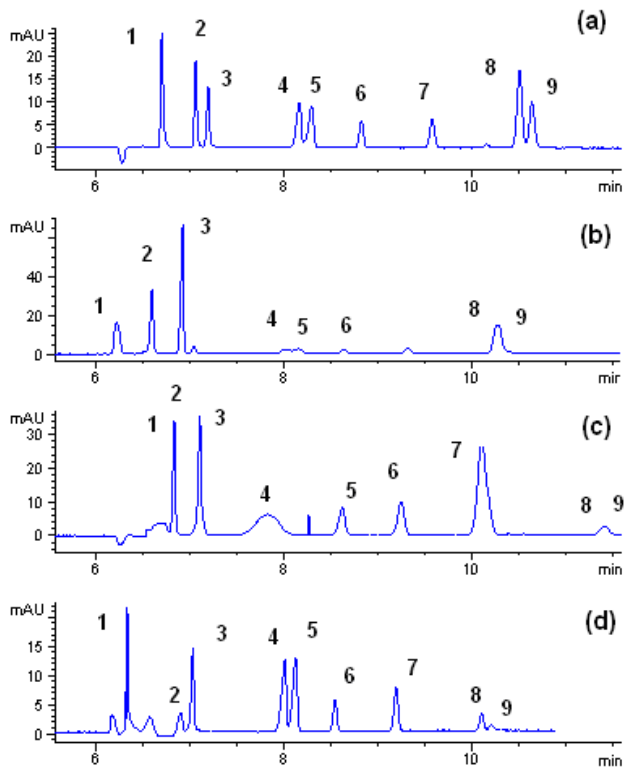

Fig.4 Effect of sample diluent on the enrichment of nine nucleoside compounds: (a) 10 mM borate buffer; (b) the microemulsion; (c) 0.1 mM NaOH; (d) methanol. Other conditions were same as in Fig. 6. Peaks: 1, cytidine (40.0 μg/mL); 2, guanosine (25.0 μg/mL); 3, N6-methyladenosine (50.0 μg/mL); 4, fluorouracil (50.0 μg/mL); 5, thymine (40.0 μg/mL); 6, adenine (175 μg/mL); 7, mercaptopurine (25.0 μg/mL); 8, 6-hydroxypurine(65.0 μg/mL); 9, guanine (40.0 μg/mL).

The injection time was investigated in the range of 5-30 s. Although prolonged time increase the sample amount, the peak shape and resolution deteriorated while the injection exceeded 10 s. The electrokinetic injection condition was also optimized by varying the injection voltage ranging +14-24 kV for 10 s. As expected, the higher injection voltage provided larger amount of sample injection enhancing the response. Whereas the voltage exceeding +22 kV broadened peak shape and decreased the resolution. The reasons may be that: (1) A overloading injection voltage led to a overloading injection volume (exceed 10% of the total volume of the capillary) causing peak broadening, resolution and sensitivity decreasing; (2) A overloading injection voltage led to an increasing Joule heating generated in sample plug, which finally affect the stability of the current and baseline of CE separation; (3) A overloading injection voltage led to unpredictable bubbles in the system bringing conductance differences between sample diluents and running buffer. Taking the amount of injection and the resolution into consideration, +22 kV×10 s was selected as the best injection condition in FASI.

Compared with normal electrokinetic injection, nine nucleoside compounds were well separated and the obtained enrichment factor was in the range of 4–35 in optimized FASI-MEEKC conditions. The optimal Electropherogram of nine nucleosides was shown in Fig.5.

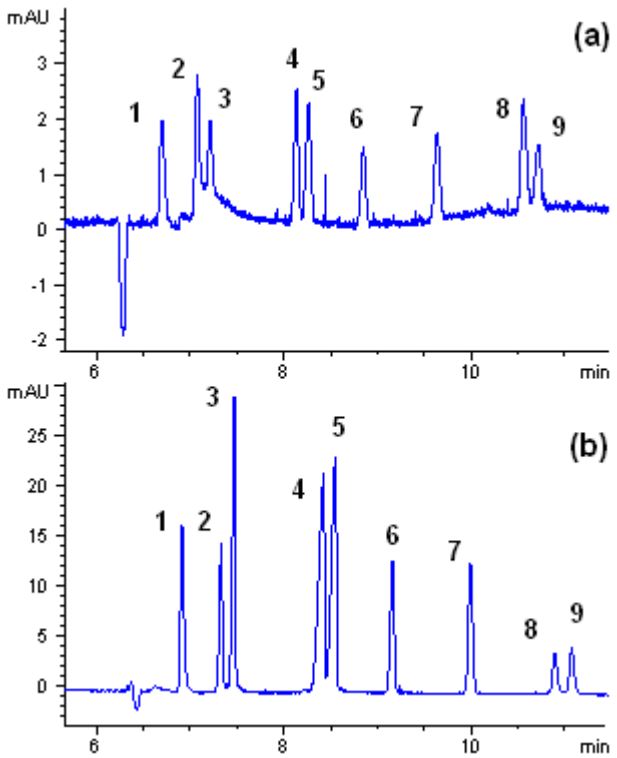

Fig.5 Comparison of electropherograms between normal electrokinetic injection and FASI: (a) Normal electrokinetic injection: +10 kV for 6 s; Peaks: 1, cytidine (4. 00 μg/mL); 2, guanosine (4.00 μg/mL); 3, N6-methyladenosine (5.00 μg/mL); 4, fluorouracil (10.0 μg/mL); 5, Thymine (8.00 μg/mL); 6, Adenine (60.0 μg/mL); 7, mercaptopurine (10.0 μg/mL); 8, 6-hydroxypurine(8.00 μg/mL); 9, Guanine (4.00 μg/mL). (b) FASI: 22 kV for 10 s; Peaks: 1, cytidine (2. 00 μg/mL); 2, guanosine (2.00 μg/mL); 3, N6-methyladenosine (2.50 μg/mL); 4, fluorouracil (5.00 μg/mL); 5, thymine (4.00 μg/mL); 6, adenine (30.0 μg/mL); 7, mercaptopurine (5.0 μg/mL); 8, 6-hydroxypurine (4.00 μg/mL); 9, guanine (2.00 μg/mL). Other conditions were the same as Fig. 3.

Method Validation

Once the method had been established, completely study of linearity, detection limit and reproducibility of this FASI-MEEKC method was conducted by analysis of a series of standard mixtures and the data were summarized in Table 1. The calibration curves of these nine analytes exhibited good linearity with R^2 in the range of 0.9915–0.9951. The detection limits at S/N = 3 were between 0.22 and 2.97 μg/mL.

To examine the precision of the proposed method, five continuous injections of a standard mixture solution with the concentration of 2.0 μg/mL for cytidine, 2.0 μg/mL for guanosine, 2.5 μg/mL for N6-methyladenosine, 5.0 μg/mL for fluorouracil, 4.0 μg/mL for thymine, 30.0 μg/mL for adenine, 5.0 μg/mL for mercaptopurine, 4.0 μg/mL for 6-hydroxypurine

and 2.0 μg/mL for guanine were analyzed. The RSDs of peak highs are in the range of 2.09–5.74% and the RSDs of the retention time vary from 0.45 to 1.12%.

Table 1. Regression equation, linearity, detection limits and repeatability of the proposed method for the analysis of nine nucleoside compounds

Compound	Regression equation	R^2	Linear range (μg/mL)	Detection limit (μg/mL)	RSD (n=5) (%)	
					Retention time (s)	Peak high (mAU)
cytidine	y=1.5118x+141.6	0.9936	1.22~75.0	0.41	0.89	3.18
guanosine	y=1.9214x+77.817	0.9937	0.65~50.0	0.22	0.68	5.74
N6-methyladenosine	y=0.9553x+80.454	0.9931	2.04~100	0.68	0.91	3.04
fluorouracil	y=1.2574x+54.756	0.9944	2.04~100	0.68	0.88	5.05
thymine	y=0.4199x+69.867	0.9920	1.22~75.0	0.41	1.05	2.09
adenine	y=0.1317x+73.636	0.9951	8.92~350	2.97	1.12	3.43
mercaptopurine	y=0.6143x+67.26	0.9935	0.65~50.0	0.22	0.65	2.98
6-hydroxypurine	y=1.4992x+19.185	0.9920	2.55~125	0.85	0.45	4.32
guanine	y=1.7018x+16.281	0.9915	1.22~75.0	0.41	0.54	4.46

The conditions were the same as in Fig. 3. y: peak high, (mAU); x: mass concentration, μg/mL

Sample analysis and recovery

To evaluate the verification of the proposed method, urine and serum samples were analyzed. The blank urine and serum sample were respectively spiked with the standard mixture at certain concentration (cytidine, 2.0 μg/mL; guanosine, 2.0 μg/mL; N6-methyladenosine, 2.50 μg/mL; fluorouracil, 5.0 μg/mL; thymine, 4.0 μg/mL; adenine, 30.0 μg/mL; mercaptopurine, 5.0 μg/mL; 6-hydroxypurine, 4.0 μg/mL; guanine, 2.0 μg/mL) and pretreated as described previously. Fig.6 and Fig.7 respectively showed the electropherograms of the urine samples and serum samples, illustrating the analytes in the spiked samples were well separated and detected without interference of impurity peaks. Recoveries of the proposed method were further investigated by spiking different concentrations of the standard mixture into urine and serum samples. From the data displayed in Table 2 and 3, we can found that the recoveries of these analytes were in the range of 91.2-113% with the RSDs of peak highs less than 5.90% in urine sample, and in the range of 85.2-112% with the RSDs of peak areas less than 8.22 % in serum.

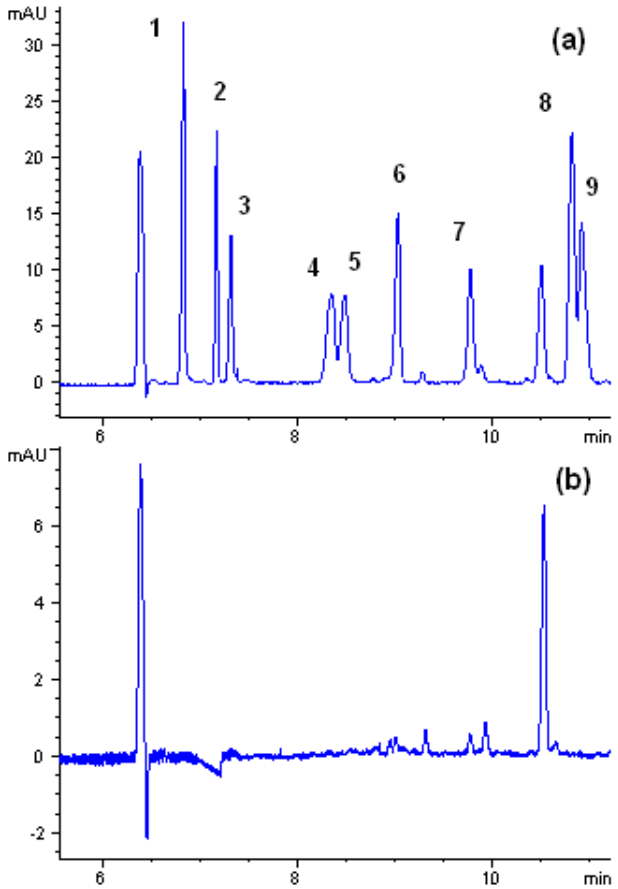

Fig.6 Electropherogram of nine nucleoside compounds in spiked urine sample (a) and blank urine sample (b). Conditions were the same as Fig. 3.

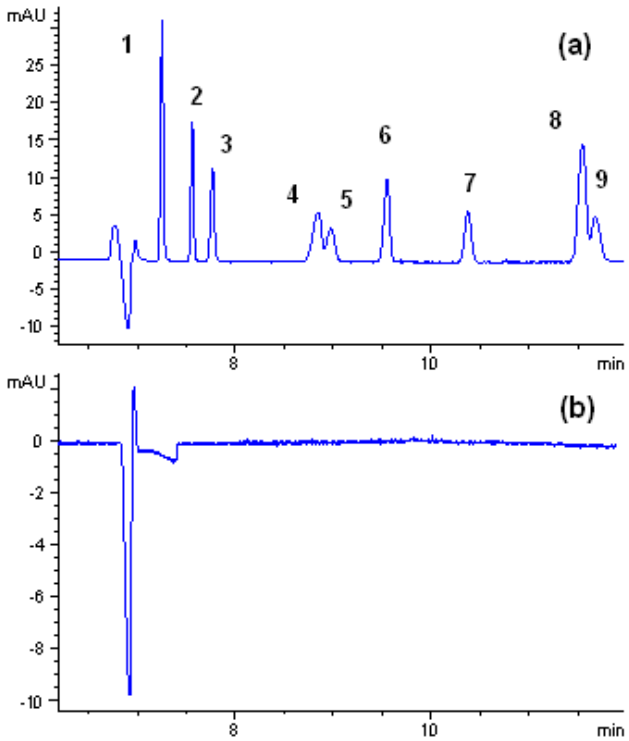

Fig.7 Electropherogram of nine nucleoside compounds in spiked serum sample (a) and blank serum sample (b). Conditions were the same as Fig. 3.

Table 2. Recovery of nine nucleoside compounds in urine sample

Compound	Added (μg/mL)	Found (μg/mL)	Recovery (%)	RSD (n=3) (%)
cytidine	50.0	45.6	91.2	2.16
	5.00	5.45	109	3.89
guanosine	30.0	29.7	99.0	4.13
	5.00	4.89	97.8	5.67
N6-methyladenosine	50.0	48.6	97.2	2.98
	10.0	9.87	98.7	4.12
fluorouracil	50.0	52.9	106	2.79
	10.0	11.3	113	3.12
thymine	50.0	48.9	97.8	2.36
	5.00	5.01	100	4.78
adenine	100	99.7	99.7	2.21
	20.0	21.5	108	3.78
mercaptopurine	30.0	32.8	109	4.13
	5.00	4.90	98.0	5.90
6-hydroxypurine	50.0	50.6	101	4.41
	10.0	9.89	98.9	5.78
guanine	50.0	51.2	102	2.25
	5.00	4.78	96.0	4.01

The conditions were the same as in Fig. 3.

Table 3. Recovery of nine nucleoside compounds in serum sample

Compound	Added (μg/mL)	Found (μg/mL)	Recovery (%)	RSD (n=3) (%)
cytidine	50.0	42.6	85.2	4.75
	5.00	5.05	101	5.39
guanosine	30.0	31.7	106	5.43
	5.00	4.57	91.4	6.66
N6-methyladenosine	50.0	49.1	98.2	5.18
	10.0	9.77	97.7	8.22
fluorouracil	50.0	55.9	112	3.99
	10.0	9.3	93.0	4.02
thymine	50.0	48.9	97.8	6.36
	5.00	5.32	105	7.58
adenine	100	96.4	96.4	5.05
	20.0	18.9	94.5	7.18
mercaptopurine	30.0	29.8	99.3	5.19
	5.00	4.44	88.0	6.67
6-hydroxypurine	50.0	47.6	95.2	6.43
	10.0	9.14	91.4	7.78
guanine	50.0	54.9	110	5.29
	5.00	5.38	108	6.31

The conditions were the same as in Fig. 3.

Conclusions

A stable, isotropically MEEKC method was on-line combined with FASI for the determination of nucleoside compounds. Herein, the MEEKC offered the rapid separation, and the FASI significantly improved the detection sensitivity of the analytes. The highly efficient and sensitive hyphenation has been successfully applied to the determination of human urine and serum. We expect that its significance for routine analysis will continue in further studies, so that it can be easily used for the monitoring the nucleoside compounds in disease diagnosis, or possibly promotes the application prospect in the nucleoside profile information natural pharmaceutical chemistry, pharmaceutical analysis.

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Reference

1. B. Feng, M. H. Zheng, Y. F. Zheng, A. G. Lu, J. W. Li, M. L. Wang, J. J. Ma, G. W. Xu, B. Y. Liu and Z. G. Zhu, *Journal of gastroenterology and hepatology*, 2005, 20, 1913-1919.
2. Y. Ma, G. Liu, M. Du and I. Stayton, *Electrophoresis*, 2004, 25, 1473-1484.
3. H. Y. Cheung, C. W. Ng and D. J. Hood, *J. Chromatogr. A*, 2001, 911.
4. L. Simeone, G. Mangiapia, G. Vitiello, C. Irace, A. Colonna, O. Ortona, D. Montesarchio and L. Paduano, *Bioconjugate chemistry*, 2012, 23, 758-770.
5. T. Nhujak, M. Srisa-art, K. Kalampakorn, V. Tolieng and A. Petsom, *J. Agric. Food Chem.* , 2005, 53, 1884-1889.
6. T. H. Senanayake, G. Warren and S. V. Vinogradov, *Bioconjugate chemistry*, 2011, 22, 1983-1993.
7. S. Wang, X. Zhao, Y. Mao and Y. Cheng, *J. Chromatogr. A*, 2007, 1147, 254-260.
8. G. Nass, *Modified Nucleosides and Cancer*, Springer-Verlag, New York, 1983.
9. G. Schoech, G. Sander, H. Topp, G. Heller-Schoch, C. W. Gehrke and K. C. Kuo, *Chromatography and Modification of Nucleosides, Part C*, Elsevier, Amsterdam, 1990.
10. H. M. Liebich, G. Xu, C. Di Stefano and R. Lehmann, *J. Chromatogr. A* 1998, 793
11. C. B. Jendresen, M. Kilstrup and J. Martinussen, *Anal. Biochem.*, 2011, 409, 249-259.
12. T. Cserha´ti and E. Forga´cs, *J. Biochem. Biophys. Methods*, 1999, 41, 21-30.
13. A. J. R. Teixeira, J. H. Gommers-Ampt, G. V. deWerken, J. G. Westra, J. F. C. Stavenuiter and A. P. J. M. de Jong, *Anal. Biochem.,* , 1993, 214, 474-483.
14. J. Ravanat, P. Guicherd, Z. Tuce and J. Cadet, *Chem. Res. Toxicol.* , 1999, 12, 802-808.
15. N. Mesplet, P. Morin, C. Francois and L. A. Agrofoglio, *Anal. Chem.*, 2008, 80, 1263-1271.
16. P. Schmitt-Kopplin and M. Frommberger, *Electrophoresis*, 2003, 24, 3837-3867.
17. Y. Q. Jiang and Y. F. Ma, *Anal. Chem.* , 2009, 81, 6474–6480
18. N. Mesplet, P. Morin, C. Francois and L. A. Agrofoglio, *J. Chromatogr. A*, 2001, 927 161–168.
19. N. Mesplet, P. Morin and L. A. Agrofoglio, *Electrophoresis*, 2002, 23 1263–1271.
20. P. Schmitt-Kopplin, *Capillary Electrophoresis Method and Protocols*, Humana Press, Totowa, 2008
21. S. Viglio, M. Fumagalli, F. Ferrari, A. Bardoni, R. Salvini, S. Giuliano and P. Iadarola, *Electrophoresis*, 2012, 33, 36-47.
22. Z. Chen, Z. Lin, L. Zhang, Y. Cai and L. Zhang, *The Analyst*, 2012, 137, 1723-1729.
23. B. Fogarty, E. Dempsey and F. Regan, *J. Chromatogr. A*, 2003, 1014, 129-139.
24. L. S. Yu, K. D. Chu, H. Z. Ye, X. X. Liu, L. S. Yu, X. Q. Xu and G. N. Chen, *TrAC*, 2012, 34, 140-151.
25. M. Geiger, A. L. Hogerton and M. T. Bowser, *Anal. Chem.*, 2012, 84, 577-596.
26. R. Ryan, S. Donegan, J. Power, E. McEvoy and K. Altria, *Electrophoresis*, 2009, 30, 65-82.
27. M. D. Mertzman and J. P. Foley, *J. Chromatogr. A*, 2005, 1073, 181-189.
28. P. Puig, F. Borrull, M. Calull and C. Aguilar, *Chromatographia*, 2005, 62, 603-610.
29. J. Zhu, S. Qi, H. Zhang, X. Chen and Z. Hu, *J, Chromatogr, A*, 2008, 1192, 319-322.
30. P. Puig, F. Borrull, C. Aguilar and M. Calull, *Journal of chromatography. B, Analytical technologies in the biomedical and life sciences*, 2006, 831, 196-204.
31. H. F. Fang, Z. R. Zeng, L. Liu and P. D. W, *Anal. Chem.* , 2006, 78 1257-1263.
32. E. Liotta, R. Gottardo, C. Seri, C. Rimondo, I. Miksik, G. Serpelloni and F. Tagliaro, *Forensic science international*, 2012, 220, 279-283.
33. X. Y. Fu, J. D. Lu and Z. A., *J. Chromatogr. A,* , 1996, 735 353-356.