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A combined solid phase extraction/capillary gel electrophoresis method for the determination of phosphorothioate oligodeoxynucleotides in biological fluids, tissues and feces

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ABSTRACT

A novel biological sample clean-up procedure has been developed for the determination of phosphorothioate oligodeoxynucleotides (PS-ODNs) and derived metabolites in biological fluids (plasma, urine and bile) and in tissues and feces from mice and rats. This method uses a one-step C18 solid-phase extraction (SPE) for biological matrix removal, and it uses capillary gel electrophoresis (CGE) for analyte detection. The assay is specific, and its linearity is superb ($r > 0.99$) for IV-AS (a 13-mer PS-ODN) and PS19 (a 19-mer PS-ODN) in a variety of biological matrices. For both IV-AS and PS19, the precision, accuracy and absolute recovery values were found to be <20%, ±20% and 80–120%, respectively. The LODs of IV-AS and PS19 were 0.6 mg/l for plasma, 0.8 mg/l for rat urine and bile, 6 μg/g for rat tissues, and 10 μg/g for rat feces, with a signal-to-noise ratio of 3 ($S/N = 3$). This method has been successfully applied to the analysis and quantitation of PS-ODNs in various biological samples arising from preclinical pharmacokinetic studies.

1. Introduction

Antisense oligonucleotides (ASOs) have been studied extensively in recent years as potential therapeutic agents, based on their specific interference with the gene expression and synthesis of target proteins [1–5]. Therapeutic ASOs currently undergoing preclinical development and clinical trials are predominantly phosphorothioate oligodeoxynucleotides (PS-ODNs). A PS-ODN, fomivirsen, was approved by the FDA in 1998 for treatment of cytomegalovirus retinitis. Following the development of antisense technology, the ability to measure PS-ODNs quantitatively and specifically in biological matrices became critical to evaluate the pharmacokinetic (PK), pharmacokinetic/pharmacodynamic (PK/PD) and toxicokinetic properties of PS-ODNs, in order to support their preclinical and clinical development.

To date, a variety of bioanalytical methods, such as capillary gel electrophoresis (CGE) [6–10], CGE–MS [11,12], capillary zone electrophoresis (CZE) [13], high-performance liquid chromatograph (HPLC) [14], liquid chromatography–mass spectrometry (LC–MS) [15–17], liquid chromatography–tandem mass spectrometry (LC–MS/MS) [18–21] and hybridization-based enzyme-linked immunosorbent assay (ELISA) [22], have been developed and used in quantitation of PS-ODNs and metabolites. Among these analytical techniques, CGE is widely employed in preclinical and clinical studies for ASOs and allows the separation of the parent compound from chain-shortened metabolites at good resolutions and reasonable sensitivities. However, one common problem in developing CGE methods for quantitating PS-ODNs is the need for rather extensive sample clean-up for the minimization of endogenous interference with ASOs during electrokinetic injection. Typical plasma and urine sample clean-up procedures for CGE analysis involve two-step SPE followed by additional desalting via dialysis, while the sample preparation from tissue and cellular fractions normally requires liquid/liquid extraction prior to analysis, and additional clean-up with solid-phase extraction for CGE analysis [6–15,23,24]. Recently, many attempts have been made to simplify and speed up these laborious sample clean-up procedures. One successful development has been the use of one-step SPE to replace the two-step SPE method. Zhang et al. [14] reported an assay using HPLC combined with one-step SPE and liquid–liquid extraction for quantitation of PS-ODNs in human plasma, and Wei et al. [23] illustrated the application of the assay to exonuclease enzyme solutions, urine, and tissue homogenate samples. However, it was not reported that this one-step SPE method could be combined with CGE for the quantitation of PS-ODNs in tissues and biological fluids. Chen et al. [24] reported a combined HPLC–CGE method for the determina-

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Table 1
The sequence of PS-ODNs used in the study.

PS-ODNS	Sequence 5′ → 3′
IV-AS	CCTTGTTTCTACT
IS17	AGCTCCTTGTTTCTACT
PS19	GGGTTGTCTTGTGCAGTTG
IS23	AGCTGGGTTGTCTTGTGCAGTTG

tion of PS-ODNs in plasma, urine and tissue homogenates. In this study, we developed a simple, economical and robust method of quantitating PS-ODNs in various biological samples, using CGE in combination with general silica-based solid phase cartridges. For the first time, we report the determination PS-ODNs in rat bile samples.

Here, a 13-mer PS-ODN (denoted as IV-AS) was studied as the model PS-ODN, and it is designed to target the 5′-terminal conserved region of eight influenza A viral RNAs. It shows broad anti-influenza virus activities both in vitro and in vivo [1,25–27]. We also show that that this assay is applicable for the detection and quantitation of IV-AS in plasma, urine, bile, and feces, as well as in tissue homogenates from mice or rats.

2. Experimental

2.1. Chemicals and instruments

IV-AS, an internal standard (IS17, a 17-mer PS-ODN), a 19-mer PS-ODN (PS19) and another internal standard (IS23, a 23-mer PS-ODN) were synthesized on ÄKTA oligopilot II synthesizer (Uppsala, Sweden) in our laboratory. The oligomers were purified using anion-exchange HPLC and reversed-phase HPLC (Milford, MA, USA) and were determined to be greater than 98% full-length by CGE. The sequences of all PS-ODNs used in the study are included in Table 1. Tetraethyl ammonium acetate tetra hydrate (TEAA) and tetrabutyl ammonium hydrogen sulfate (TBAS) were purchased from the Sigma Chemical Company (St. Louis, MO, USA). eCAP™ ssDNA 100-R kit was purchased from Beckman Instruments, Inc. (Fullerton, CA, USA). Desalting filters with pore sizes of 0.025 μm were obtained from Millipore (Bedford, MA, USA). AccuBOND II ODS-C18 solid phase extraction cartridges were purchased from Agilent Technologies (UK). All other chemicals and reagents were of analytical grade.

2.2. Animal

Experimental animals used were male Balb/c mice (18–20 g) and Wistar rats (180–220 g) from Vital River Lab Animal Technology Co., Ltd. (Beijing, PRC). The plasma, tissue, urine, feces and bile samples from mice or rats were stored at −20 °C until analysis.

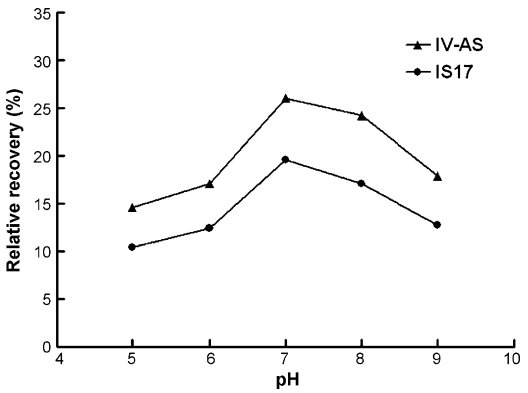

Fig. 1. Effect of Tris–HCl buffer pH on the extraction efficiency of IV-AS and IS17.

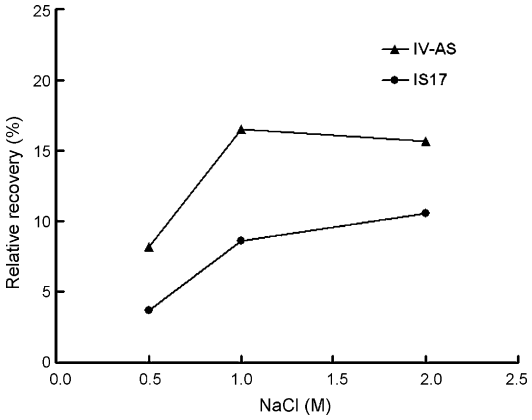

Fig. 2. Effect of NaCl concentration on the extraction efficiency of IV-AS and IS17.

2.3. Sample preparation

2.3.1. Preparation of calibration standards

IV-AS stock solutions (1000 mg/l) were made by dissolving 100 mg of IV-AS in 100 ml of deionized water. IS17 stock solutions (500 mg/l) were prepared by dissolving 50 mg of IS17 in 100 ml of deionized water. Aliquots of the stock solutions were stored at −20 °C. IV-AS calibration standards were prepared by adding a known amount of IV-AS and IS17 to biological matrices untreated with PS-ODNs, so that IV-AS nominal concentrations were 2, 10, 50, 100, 200, 400, 800, 1200 mg/l for mouse or rat plasma; 6, 150, 300, 750, 1500 μg/g for rat lung tissue; 6, 75, 150, 300, 600 μg/g for rat kidney tissue; 2, 5, 10, 25, 50 mg/l for rat urine; 10, 20, 50, 100, 250, 500 μg/g for rat feces; and 1, 2, 5, 10, 25, 50 mg/l rat bile. IS17 final concentrations were 50 mg/l for plasma, urine and bile, 150 μg/g for tissue and 500 μg/g for feces. Calibration curves were plotted as the nominal concentration versus normalized area [6]. Quality control (QC) samples were prepared by mixing appropriate amounts of stock solutions and blank biological matrices, and they were stored in batches at −20 °C for the duration of validation procedure.

2.3.2. Sample extraction procedure

A known amount of IV-AS spiked with 10 μl of IS17 stock solutions was added to an aliquot of blank plasma or urine (100 μl) and diluted with 5 ml of loading buffer (10 mM Tris–HCl, pH 7 1 M

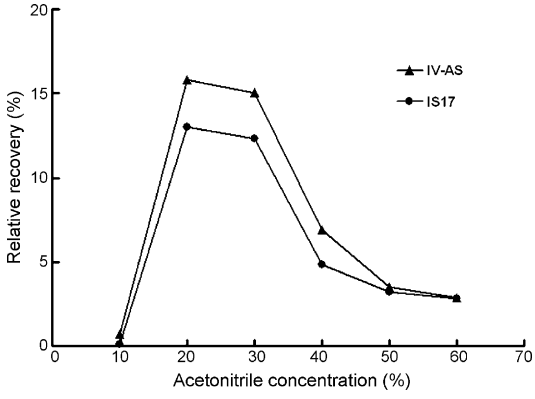

Fig. 3. Effect of acetonitrile concentration on the extraction efficiency of IV-AS and IS17.

NaCl). The tissues were weighed and homogenized in a 3-fold volume of buffer (10 mM Tris–HCl, pH 7.8, 0.01 M EDTA, 0.5% SDS) using a PRO 200 homogenizer (Oxford, CT, USA) prior to the addition of 10 μl of IS17 stock solutions. Each homogenate (500 μl) was incubated with 5 μl of 20 mg/ml proteinase K at 55 °C for 1 h to digest proteins and then extracted with 0.5 ml of phenol:isoamyl alcohol:chloroform (24:1:25) for 10 min. After centrifugation at 8000 × *g* for 15 min, the supernatant was removed and diluted with 5 ml of loading buffer (10 mM Tris–HCl, pH 7, 1 M NaCl, 10% acetonitrile). Dry feces samples were ground and weighed prior to being homogenized in a 10-fold volume of water. An aliquot of feces (500 μl) was spiked with 10 μl of IS17 stock solutions and extracted using 0.5 ml of phenol:isoamyl alcohol:chloroform (24:1:25) for 10 min. After centrifugation at 8000 × *g* for 15 min, the supernatant was removed and diluted with 5 ml of loading buffer (10 mM Tris–HCl, pH 7, 1 M NaCl, 10% acetonitrile). Each bile sample (100 μl) was spiked with 10 μl of IS17 stock solution and 200 μl of loading buffer (10 mM Tris–HCl, pH 7, 1 M NaCl, 10% acetonitrile) and extracted using 0.5 ml of phenol:isoamyl alcohol:chloroform (24:1:25) for 10 min. After centrifugation at 8000 × *g* for 15 min, the supernatant was removed and diluted with 5 ml of loading buffer. Reversed-phase C18 SPE columns were pre-equilibrated by sequential washing with 1 ml of acetonitrile, 1 ml of deionized water, 1 ml of acetonitrile, 1 ml of buffer (10 mM Tris–HCl, pH 7, 1 M NaCl), 1 ml of acetonitrile and 3 ml of loading buffer. Samples, prepared as described above, were loaded into the reversed-phase C18 SPE columns and washed with 5 ml of deionized water. IV-AS was eluted using at least 3 ml of freshly prepared 20% acetonitrile, dried in a Savant speed-vac (Holbrook, NY, USA) and resuspended in 50 μl of deionized water. The samples were dialyzed on a 0.025 μm membrane for 30 min by floatation on deionized water.

2.4. Capillary gel electrophoresis analysis

Capillary gel electrophoresis analysis was accomplished with a Beckman P/ACE™ MDQ using a 31-cm coated capillary (effective length, 20 cm) and a Beckman eCAP™ ssDNA 100-R kit. Samples were electrokinetically injected with a field strength of −10 kV for the duration of 1–30 s. Separation was achieved by running the gel at 40 °C with an applied voltage of 15.5 kV (500 V/cm) and a running current of 11–15 μA. Detection was via ultraviolet absorption at 254 nm. Areas under the curve for PS-ODN peaks were determined using the P/ACE system MDQ Software (Version 2.3). Integration parameters were a threshold of 100, a shoulder sensitivity of 100 and a peak width of 0.1. The concentrations of IV-AS in biological samples were calculated by linear interpolation using our linear calibration curves.

2.5. Method validation

In order to evaluate the suitability of the developed extraction method for the quantitation of IV-AS in mouse or rat plasma, rat lung or kidney tissues, urine, feces or bile, the specificity, the linearity, the LLOQ, the LOD, the accuracy, the precision and the absolute recovery rate of the assay were investigated at low, medium and high concentrations, and five QC samples at each concentration were tested. Furthermore, the stability of IV-AS in mouse plasmas and in standard solutions was evaluated. The assay also evaluated the suitability of PS19 (IS23 was used as an internal standard for quantitating PS19) in mouse plasma samples in order to broaden the applicability of the method mentioned above to include PS-ODNs with different base lengths.

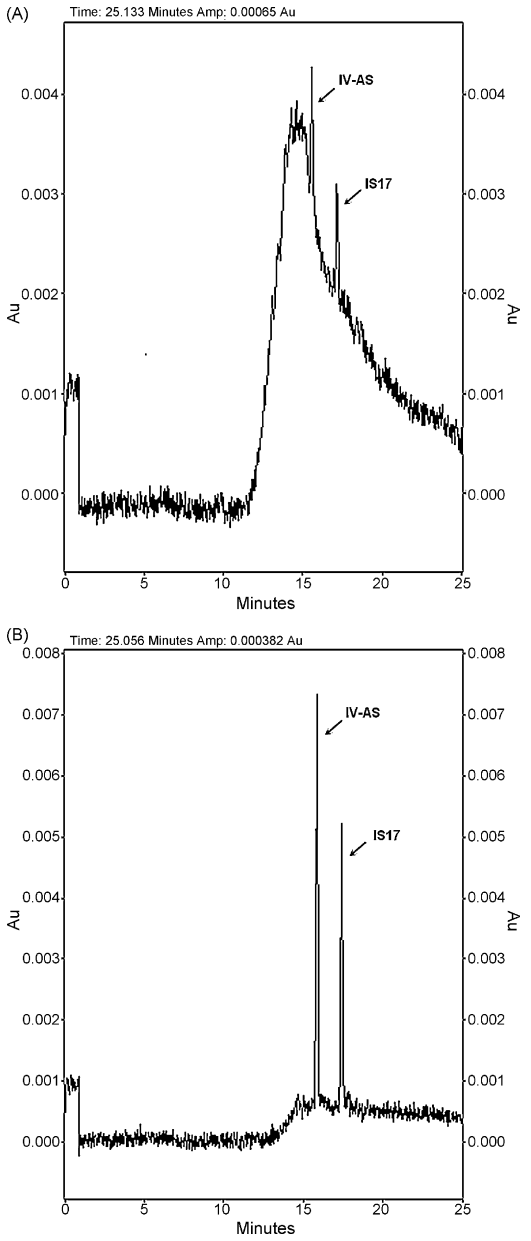

Fig. 4. CGE electropherograms of a blank lung sample spiked with IV-AS and IS17 at 150 μg/g using no acetonitrile extraction (A); a blank lung sample spiked with IV-AS and IS17 at 150 μg/g using 10% acetonitrile extraction (B).

2.6. Optimization of SPE and chromatographic conditions

SPE extraction conditions were evaluated at nominal mouse plasma concentrations of 50 mg/l for IV-AS and 50 mg/l for IS17, respectively. The relative recovery of SPE was determined by comparing the corrected areas (the quotient of the peak area of processed samples divided by the migration time) of processed samples with those of initial standard solutions (50 mg/l) [6,24]. The processed samples were prepared by the extraction procedure described in Section 2.3.2 except for varying loading buffer pH values at 5, 6, 7, 8 and 9; NaCl concentrations at 0.5, 1, and 2 M; and the acetonitrile percentage of elution solution at 10, 20, 30, 40, 50 and 60%, respectively. Capillary gel electrophoresis analysis was as described in Section 2.4, except that electrokinetic injection was for 10 s at −10 kV.

Table 2
The accuracy, precision and absolute recovery rates of PS-ODNs (IV-AS, PS19) in mouse plasma.

	IV-AS			PS19		
Range (mg/l), *r*		2–1200, 0.9990			2–800, 0.9994	
LLOQ (mg/l), R.S.D.%, R.E.%		2, 16.0, −9.3			2, 6.8, 6.0	
LOD (mg/l) (*S*/*N* = 3)		0.6			0.6	
Nominal concentration (mg/l, *n* = 5)	5	200	1200	5	200	800
Precision (R.S.D.%)[a]						
Within-day	3.9	2.5	4.4	1.7	3.8	6.7
Between-day	4.9	4.4	13.6	2.7	5.3	7.0
Accuracy (R.E.%)[b]	−0.2	11.2	3.7	−2.5	2.6	−2.4
Absolute recovery rate (%)[c]	99.8 ± 5.1	111.2 ± 4.9	103.7 ± 13.2	97.5 ± 2.3	102.6 ± 5.4	97.6 ± 6.8

n, the number of replicate samples.
[a] R.S.D.%, relative standard deviation.
[b] R.E.%, relative error. R.E.% = (observed concentration/nominal concentration − 1) × 100.
[c] The absolute recovery rate is calculated as follows: (observed concentration/nominal concentration) × 100.

3. Results and discussion

3.1. Optimization of SPE conditions

3.1.1. Effect of loading buffer pH

IV-AS was ionized by adjusting plasma samples to different pH values with Tris–HCl buffer. As expected, IV-AS retention was affected by the pH of the buffer used (Fig. 1). IV-AS was extracted from plasma samples at pH 7, and the maximal extraction of IV-AS was obtained at a pH 7. But the extraction efficiency of IV-AS gradually decreased with increasing buffer pH higher than 7. This interesting phenomenon was not consistent with results from studies that purified PS-ODNs using a one-step ion-pairing reversed-phase SPE [14]. However, in general, silica-based SPE cartridges are suitable for use at pH 7.

3.1.2. Effect of NaCl concentration

The retention of IV-AS was also affected by the concentration of NaCl (Fig. 2). With increasing concentrations of NaCl, the retention of IV-AS by the C18 SPE column also increased. However, higher concentrations of NaCl may affect subsequent desalting steps. Therefore, we chose an optimal salt concentration of 1 M NaCl.

3.1.3. Effect of acetonitrile concentration

Besides the above factors, the IV-AS extraction was also influenced by the concentration of acetonitrile in the SPE elution solution. As shown in Fig. 3, increasing the acetonitrile concentration increased the elution of IV-AS, until acetonitrile concentrations of over 20%, after which extraction yields were lower. Thus, elution solution with 20% acetonitrile seemed to be optimal.

These effects on the extraction of IV-AS were similar to those on IS17, and the absolute recovery rate of IV-AS after one-step C18 SPE was higher than 90% in plasma samples under the optimized conditions discussed above (Table 2).

In addition to NaCl, the ion-pairing agents TEAA and TBAS were investigated in SPE. Both showed similar behavior and did not significantly extract IV-AS from plasma samples, which clearly shows that NaCl may play other important roles besides being an ion-pairing effect. It is known that PS-ODNs are “sticky” and bind nonspecifically to plasma proteins. The addition of NaCl in

Table 3
Studied ranges, LLOQs, LODs, precisions, accuracies and absolute recovery rates of IV-AS in rat plasma, lung tissue, kidney tissue, urine, feces and bile.

Matrix	Range (*r*)	LLOQ (R.S.D.%, R.E.%)	LOD (*S*/*N* = 3)	Nominal concentration	Precision (R.S.D.%) (*n* = 5)		Accuracy (%)	Absolute recovery rate (%)
					Within-day	Between-day		
Plasma (mg/l)	2–1200 (0.9989)	2	0.6	2	2.0	5.7	−2.2	97.8 ± 1.9
				100	4.9	8.4	−11.1	88.9 ± 4.3
				1200	1.4	2.0	−8.5	91.5 ± 1.9
Lung (μg/g)	6–1500 (0.9987)	6	6	6	1.7	3.6	−2.1	97.9 ± 7.0
				150	3.4	9.3	2.5	102.5 ± 10.6
				1500	2.2	2.9	−8.8	91.2 ± 12. 6
Kidney (μg/g)	6–600 (0.9958)	6	6	6	15.2	19.5	16.0	116.0 ± 28.4
				150	3.3	5.4	8.9	108.9 ± 13.8
				600	8.4	9.7	12.0	112.0 ± 14.6
Urine (mg/l)	2–50 (0.9977)	2	0.8	2	7.0	11.0	6.9	106.9 ± 16.4
				10	6.9	8.4	−12.6	87.4 ± 9.4
				50	3.3	4.0	7.2	107.2 ± 5.5
Feces (μg/g)	10–500 (0.9901)	10 (3.4, −6.6)	10	20	8.0	16.2	16.2	116.2 ± 31.9
				100	8.9	14.2	7.4	107.4 ± 12.8
				500	6.9	15.9	3.1	103.1 ± 14.9
Bile (mg/l)	1–50 (0.9907)	1 (19.6, −13.5)	0.8	2	8.5	17.9	14.6	114.6 ± 18.0
				10	1.8	3.6	−0.1	99.9 ± 5.8
				50	3.1	5.6	−0.2	99.8 ± 5.7

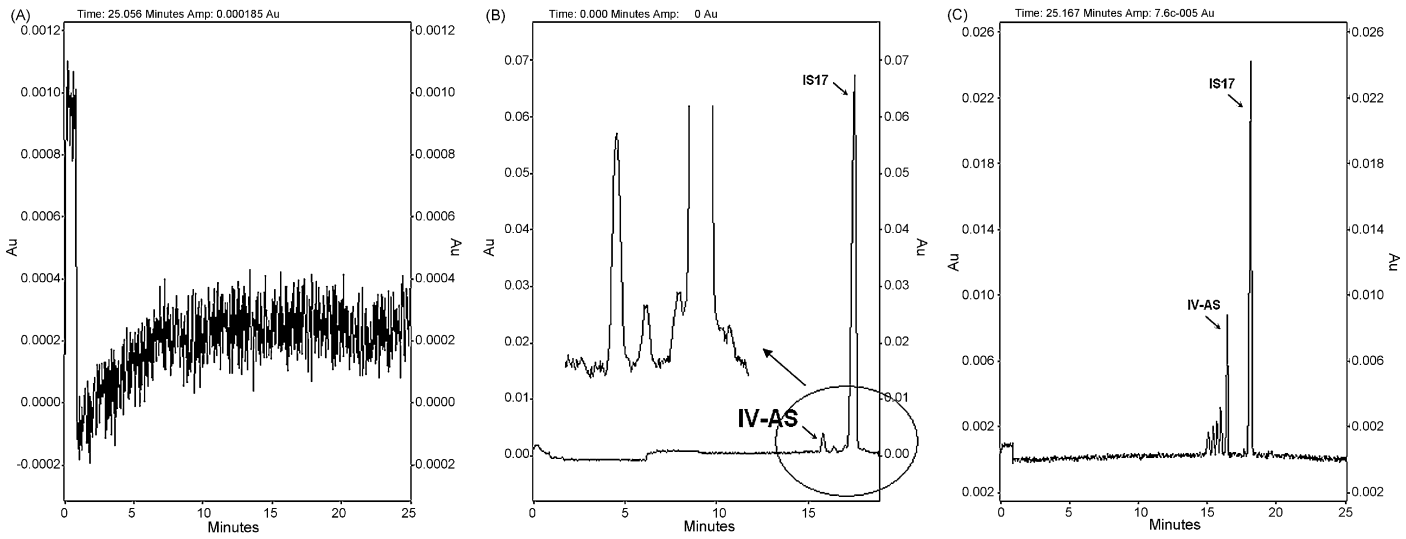

Fig. 5. CGE electropherograms of IV-AS in mouse plasma: (A) blank plasma; (B) blank plasma spiked with 2 mg/l of IV-AS and 50 mg/l IS17; (C) plasma sample at 30 min after a single intravenous bolus injection of 40 mg/kg IV-AS.

the plasma perhaps modulates simple charge-shielding effects between PS-ODNs and proteins. On the other hand, NaCl added to loading buffer may be used to adjust the sample ionic strength to strengthen the affinity for C18 SPE columns because extraction yields were minimal without use of any NaCl. Therefore, the mechanism of this clean-up approach awaits further explanation.

3.2. Rat plasma, tissues, urine, feces and bile sample extraction

The preparation of rat plasma and urine samples was similar to that of mouse plasma samples, while tissue, bile and feces samples differed only in the constituents of the loading buffer and of the liquid–liquid extraction. The above extraction method was modified by adding 10% acetonitrile to the loading buffer for tissue samples. Blank rat lung samples spiked with IV-AS and IS17 at 50 mg/l were extracted using either the unmodified or modified SPE method. It can be seen from Fig. 4A and B that the addition of 10% acetonitrile obviously reduced the interference from endogenous substances in the lung sample. The modified method was also applied to bile, feces and other tissue samples, such as kidney, liver and so on.

3.3. Assay evaluation

The specificity of the method was evaluated using blank mouse plasma samples from six different mice and blank rat biological samples from five different rats. CGE electropherograms of blank biological samples, blank biological samples spiked with IV-AS and IS17, and biological samples after administration are shown in Figs. 5–11. SPE removed the endogenous constituents of the biological samples and thus did not interfere with quantitation of IV-AS.

Tables 2 and 3 show the linear range, the LLOQ (lower limit of quantitation), the LOD (limit of detection), the precision, the accuracy and the absolute recovery rate data for IV-AS or PS19 in plasma, tissue, urine, feces and bile samples. Over the studied concentra-

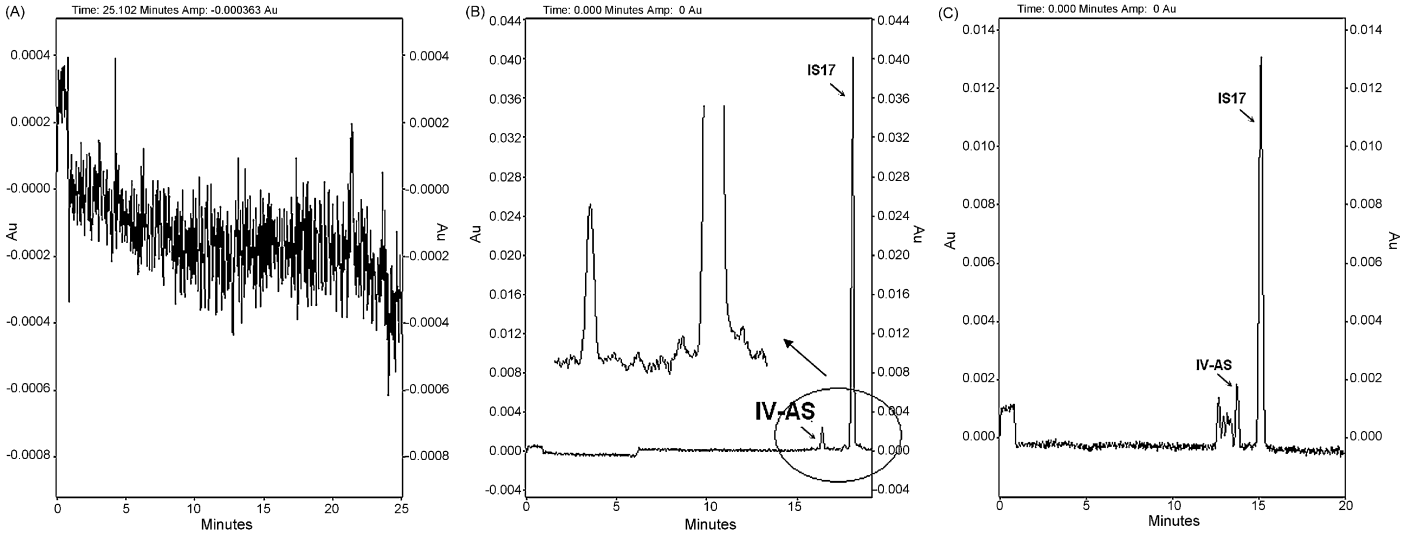

Fig. 6. CGE electropherograms of IV-AS in rat plasma: (A) blank plasma; (B) blank plasma spiked with 2 mg/l of IV-AS and 50 mg/l IS17; (C) plasma sample at 6 h after an intrapulmonary infusion of 20 mg/kg IV-AS.

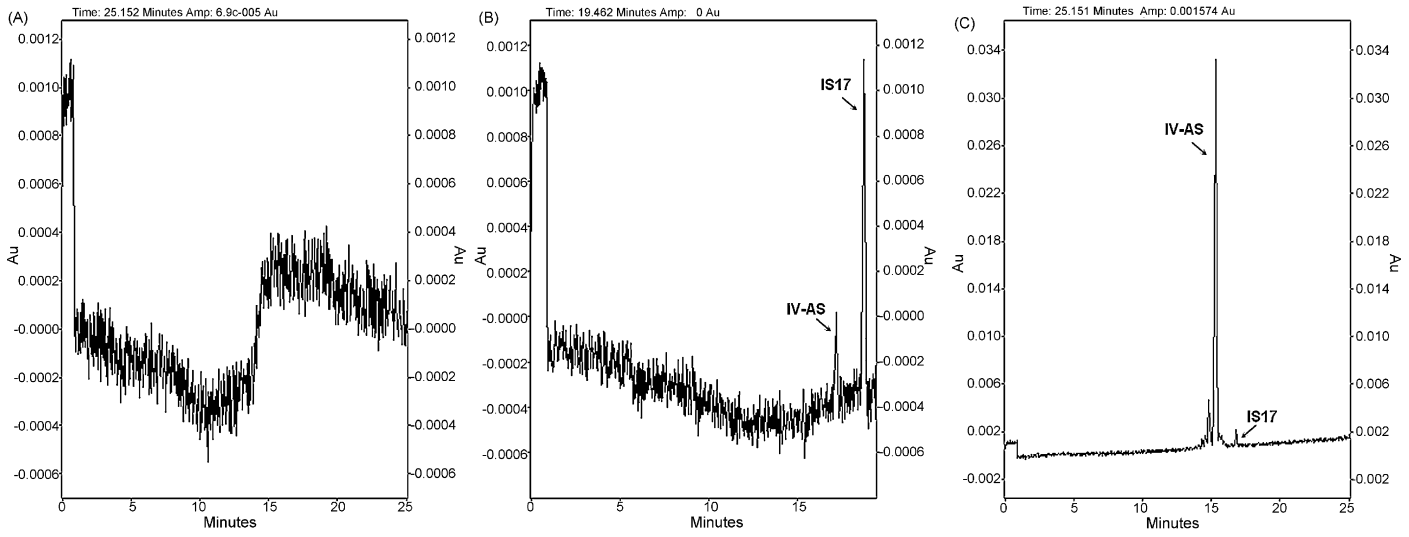

Fig. 7. CGE electropherograms of IV-AS in rat lung: (A) blank lung; (B) blank lung spiked with 6 μg/g of IV-AS and 150 μg/g IS17; (C) lung sample at 6 h after an intrapulmonary infusion of 20 mg/kg IV-AS.

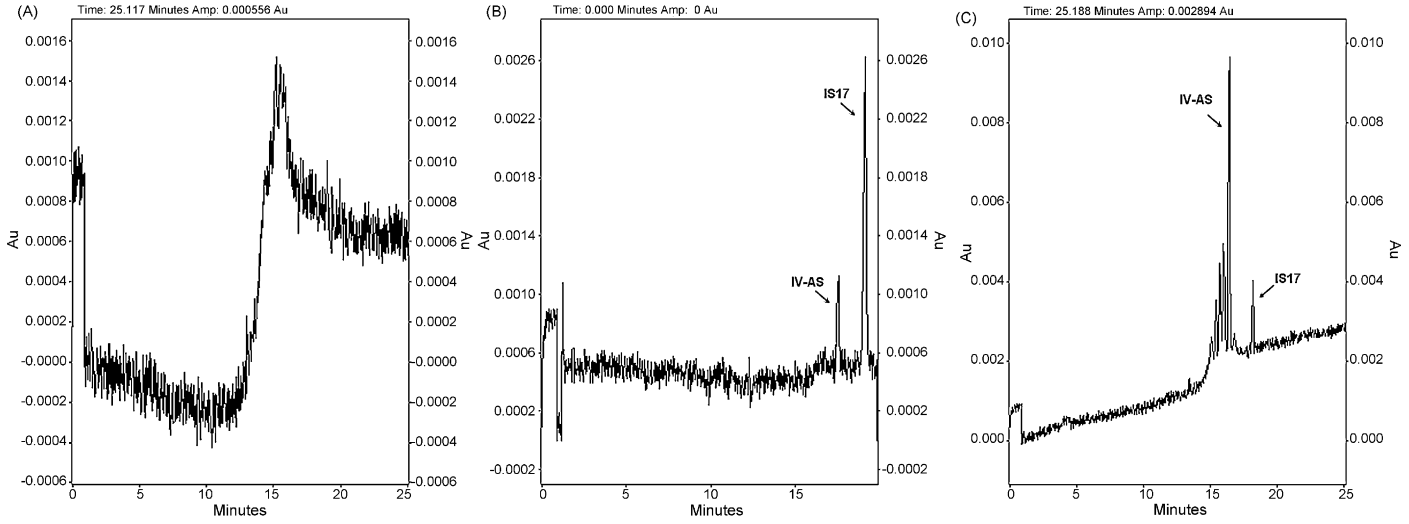

Fig. 8. CGE electropherograms of IV-AS in rat kidney: (A) blank kidney; (B) blank kidney spiked with 6 μg/g of IV-AS and 150 μg/g IS17; (C) kidney sample at 6 h after an intrapulmonary infusion of 20 mg/kg IV-AS.

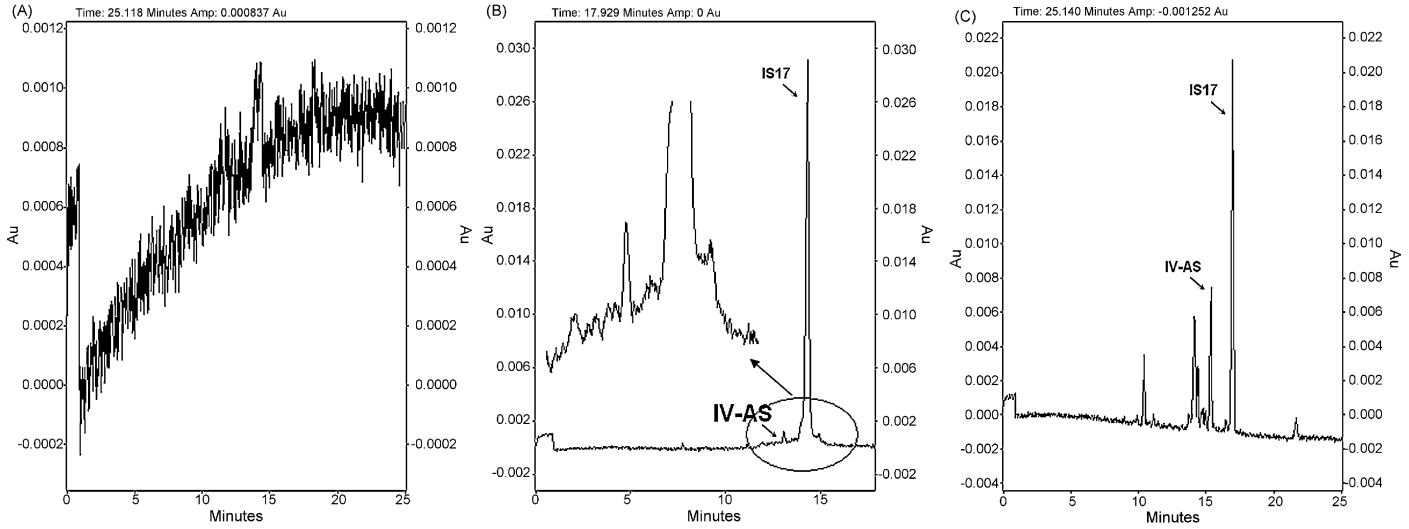

Fig. 9. CGE electropherograms of IV-AS in rat urine: (A) blank urine; (B) blank urine spiked with 2 mg/l of IV-AS and 50 mg/l IS17; (C) urine sample collected from 0 to 5 h after an intrapulmonary infusion of 20 mg/kg IV-AS.

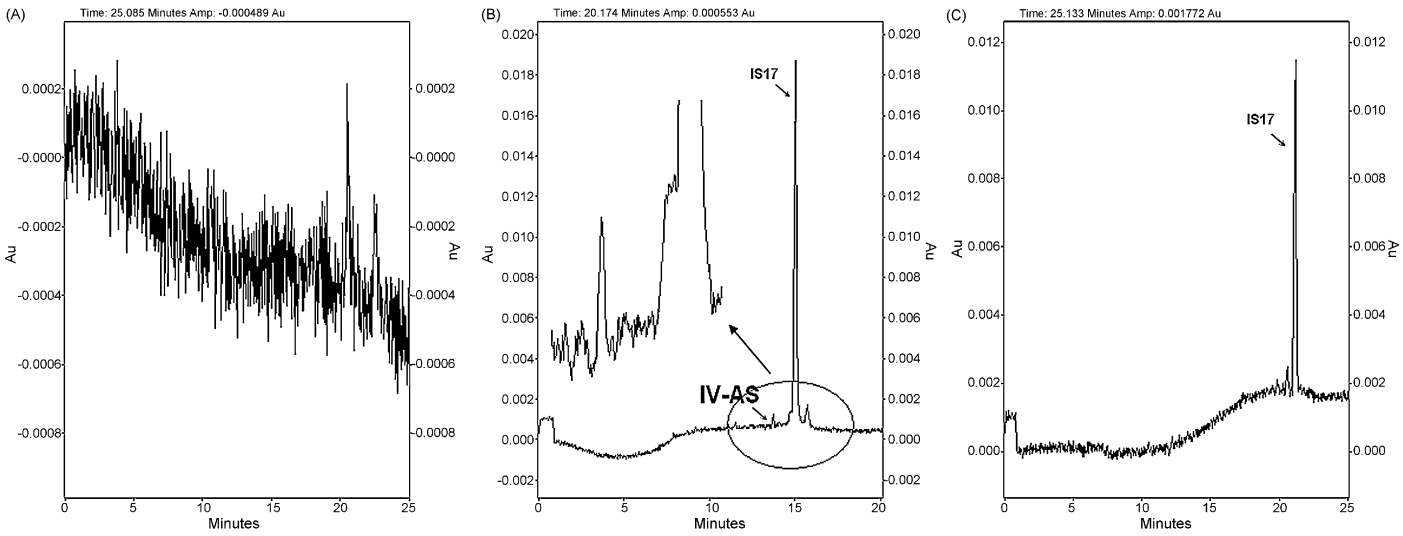

Fig. 10. CGE electropherograms of IV-AS in rat feces: (A) blank feces; (B) blank feces spiked with 10 μg/g of IV-AS and 500 μg/g IS17; (C) feces sample collected from 0 to 12 h after an intrapulmonary infusion of 20 mg/kg IV-AS.

tion ranges, the correlation coefficients (r) of calibration curves for IV-AS or PS19 in different biological matrices were excellent. The precision of the analysis was evaluated from the within-day and between-day variability of QC samples at three different concentrations, and it is expressed as the relative standard deviation (R.S.D.%). The between-day and within-day precision values ranged from 1.4 to 19.5% in biological matrices for IV-AS and 1.7–7.0% in mouse plasma for PS19. The accuracy was measured as the difference between the actual to predicted concentrations and expressed as the relative error (R.E.%). The accuracy of QC samples for IV-AS ranged between −12.6 and 16.2% in biological matrices, and for PS19, it ranged between −2.5 and 2.6% in mouse plasma. The absolute recovery rates of QC samples for IV-AS and PS19 in plasma, lung, kidney, urine, feces and bile ranged from 87.4 to 116.2%. The LLOQs of IV-AS and PS19 were assessed as the lowest concentration used in calibration curves in the different biological matrices. Accuracy and precision were less than 20% at LLOQ. The LODs of IV-AS and PS19 were 0.6 mg/l for plasma, 0.8 mg/l for rat urine and bile, 6 μg/g for tissues, and 10 μg/g for feces, with a signal-to-noise ratio of 3 ($S/N = 3$). These values validate that this assay is reproducible and reliable.

Also, the stability of IV-AS in mouse plasma was evaluated using QC samples stored at normal laboratory conditions for 2 h, at −20 °C for 30 d, and after subjection to two full cycles of freeze-thaws. The stability of QC sample solutions after extraction as prepared above was evaluated after storage at room temperature for 24 h and at 4 °C for 14 d. In addition, the stability of IV-AS standard solution was evaluated after storage at 4 °C for 1, 7 and 14 d and −20 °C for 1, 3, 6 and 12 months. It was found that there was no significant degradation for IV-AS in mouse plasma samples or standard solutions. Hence, the plasma samples were shown to be stable under the above storage conditions.

3.4. Method application

Fig. 12 shows the plasma concentration–time profile following a single intravenous bolus dose of 40 mg/kg of IV-AS to mice. Following injection, IV-AS was rapidly distributed to the tissues and cleared from the blood, with an elimination half-life of about 1 h. As shown in Figs. 5C–11C, after intrapulmonary administration of 20 mg/kg of IV-AS to rats, deposition and clearance half-lives of IV-AS in the major organs (lung and kidney) were much higher than

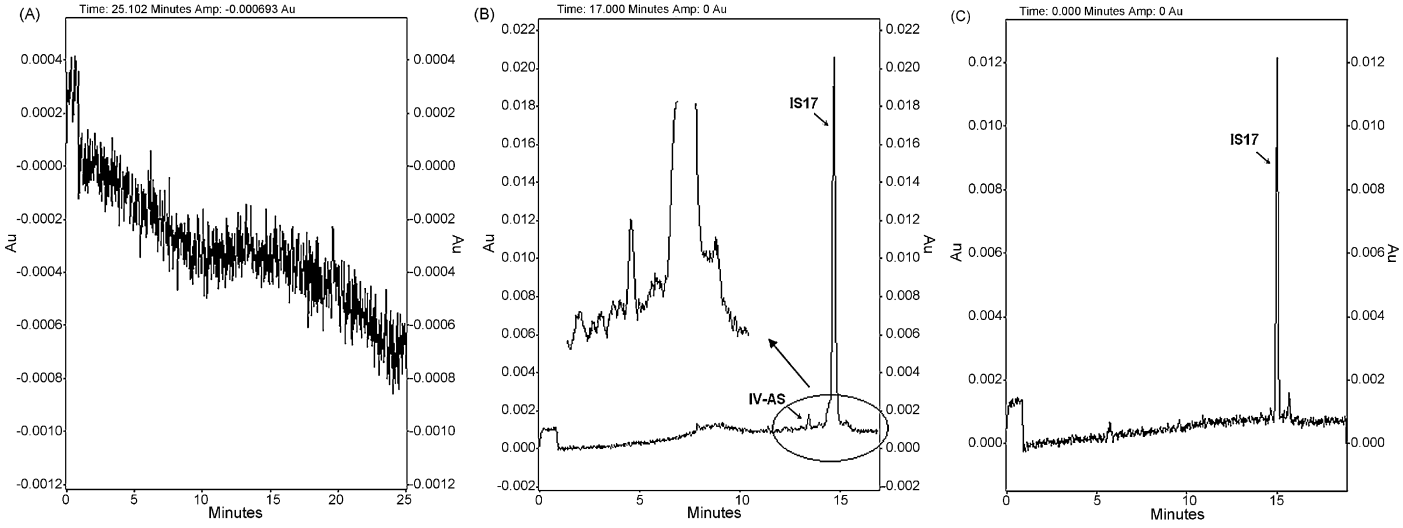

Fig. 11. CGE electropherograms of IV-AS in rat bile: (A) blank bile; (B) blank bile spiked with 1 mg/l of IV-AS and 50 mg/l IS17; (C) bile sample collected from 0 to 5 h after an intrapulmonary infusion of 20 mg/kg IV-AS.

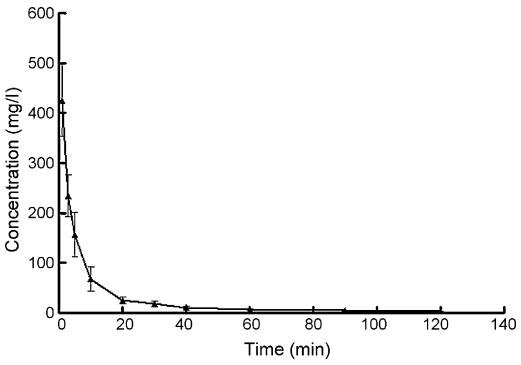

Fig. 12. Plasma concentration–time profile following a single intravenous bolus doses of 40 mg/kg of IV-AS to mice. Six animals were used for each time point.

in plasma. Very little of the intact IV-AS was excreted from urine or feces in the first 12 h. The concentrations of IV-AS in bile samples were not detected during the first 5 h. The PK of IV-AS was thus similar to that of various reported PS-ODNs in preclinical and clinical trials [28–31].

4. Conclusion

This method for quantitation of IV-AS and PS19 in biological matrices is simple and reliable. By optimizing experimental conditions, a novel one-step SPE method was established, and the cost of sample handling was significantly reduced. In addition, this modified preparation method was successfully applied in measuring plasma, tissue, urine, feces and bile samples from mice or rats, and it provided for a relatively complete characterization of the ADME (absorption, distribution, metabolism and excretion) properties of PS-ODNs in preclinical pharmacokinetic studies. Furthermore, this assay could be used to extract PS-ODNs of different base lengths in various biological samples. The LLOQ and LOD of the current method are similar to those of previously reported CGE methods [7,10], but not as low as the LC–MS/MS methods or hybridization-based ELISAs. Therefore, this assay constitutes an alternative for quantitating oligodeoxynucleotides from biological samples.

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