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Note

Effect of sodium dodecyl sulphate on the extraction of ubiquinone-10 in the determination of plasma samples

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The determination of plasma ubiquinone-10 (UQ-10, the number ten indicating the number of isoprenoid side-chains) has been reported in connection with human bioavailability studies of UQ-10 [1, 2]. The most commonly used method is based on the extraction of UQ-10 from the plasma sample with *n*-hexane followed by analysis of the extract on a reversed-phase high-performance liquid chromatographic (HPLC) column. In the course of our bioavailability studies, we encountered difficulty with low sensitivity and poor reproducibility of the determination, which resulted from the low recovery of UQ-10 from the sample. We found that the low recovery was associated with the low temperature of our laboratory during the winter season.

This paper reports the finding that the addition of sodium dodecyl sulphate (SDS) to the plasma sample before extraction with the hexane significantly improves the recovery at low ambient temperatures.

EXPERIMENTAL

Reagents and apparatus

UQ-10 was received as a gift from Nisshin Chemicals (Tokyo, Japan). Other reagents and solvents were of the best commercially available grades. HPLC

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was carried out on a Shimadzu liquid chromatograph, Model 3A. UQ-10 was detected at 275 nm with an ultraviolet (UV) detector. Separation was obtained on a Zorbax ODS column (15 × 0.46 cm; particle size 5—6 μm) with a mobile phase consisting of 96% ethanol in water. The flow-rate was 1.0 ml/min at 54°C.

Determination

To a human plasma sample (1.0 ml) was added a solution (1.0 ml) of 200 m*M* SDS, and the mixture was shaken vigorously to obtain an emulsion. After the addition of methanol (3.0 ml) UQ-10 was extracted with *n*-hexane (5.0 ml).

TABLE I

EFFECT OF TEMPERATURE ON EXTRACTION OF PLASMA UQ-10 WITH HEXANE

Temperature (°C)	Additive	UQ-10 added (μg/ml plasma)	UQ-10 found (μg/ml plasma)			Recovery (%)		C.V. (%)
			Value		Mean found	Value	Mean ± S.D.	
7	None	0.0	0.13	0.15	0.14	—		
		0.5	0.18	0.18	0.18	8.0		
		1.0	0.25	0.33	0.29	15.0	12.3 ± 3.10	25.20
		1.5	0.33	0.36	0.35	14.0		
		2.0	0.39	0.36	0.38	12.0		
	SDS	0.0	1.09	1.06	1.08	—		
		0.5	1.53	1.58	1.56	96.0		
		1.0	2.09	2.09	2.09	101.0	100.9 ± 4.09	4.05
		1.5	2.59	2.59	2.59	100.7		
		2.0	3.17	3.22	3.20	106.0		
17	None	0.0	0.96	0.98	0.97	—		
		0.5	1.41	1.39	1.40	86.0		
		1.0	1.76	1.85	1.81	84.0	86.4 ± 2.06	2.38
		1.5	2.23	2.30	2.27	86.7		
		2.0	2.81	2.69	2.75	89.0		
	SDS	0.0	1.03	1.03	1.03	—		
		0.5	1.50	1.52	1.51	96.0		
		1.0	1.94	1.99	1.97	94.0	95.2 ± 0.99	1.04
		1.5	2.44	2.45	2.45	94.7		
		2.0	2.95	2.95	2.95	96.0		
30	None	0.0	1.02	1.07	1.04	—		
		0.5	1.48	1.56	1.52	96.0		
		1.0	1.96	1.99	1.98	93.5	94.8 ± 1.26	1.33
		1.5	2.46	2.44	2.45	94.0		
		2.0	2.94	2.97	2.96	95.8		
	SDS	0.0	1.06	1.04	1.05	—		
		0.5	1.53	1.51	1.52	94.0		
		1.0	2.03	2.00	2.02	97.0	95.6 ± 1.25	1.31
		1.5	2.49	2.48	2.49	96.0		
		2.0	2.99	2.93	2.96	95.5		

The hexane phase (4.0 ml) was removed and evaporated. The resulting residue was dissolved in the HPLC mobile phase (1.0 ml) at 70°C, and was analysed on the HPLC column.

The concentration of UQ-10 in plasma was obtained directly from a calibration curve obtained by analyses of control plasma samples (1.0 ml) to which known quantities (0.5—2.0 μg) of UQ-10 in ethanol (20 μl) were added. The curve was prepared from peak heights corresponding to UQ-10, for which the retention time was 14 min.

RESULTS AND DISCUSSION

SDS used as an additive was included in the hexane extract; however, it did not interfere with the separation of UQ-10. Over 400 samples in our bioavailability studies were analysed on a column without deterioration of the resolution.

Samples were analysed in the presence and absence of SDS at three different temperatures. At 30°C SDS had almost no effect on the recovery of UQ-10 and coefficient of variation (C.V.). However, at 7°C and 17°C, the additive improved both the recovery and C.V., especially at 7°C (Table I). These findings indicate the necessity of using SDS when conducting this analysis at temperatures below 17°C.

The recovery of UQ-10, which was added to a control plasma in a known amount, was studied at various concentrations of SDS at 7°C (Fig. 1). Although the recovery was 47% in the absence of SDS, no recovery was obtained in 8 and 43 m*M* SDS. The presence of at least 50 m*M* SDS resulted in almost complete recovery. Therefore the determination of UQ-10 by this method included SDS at the concentration of 100 m*M*.

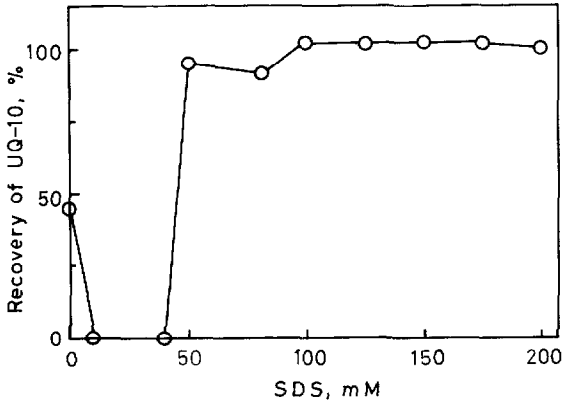

Fig. 1. Effect of SDS on recovery of UQ-10. UQ-10 (2.0 μmol) was added to control plasma (1.0 ml). SDS solutions (1.0 ml) were added to obtain plasma samples with varying concentrations of SDS. The analysis of UQ-10 was carried out at 7°C.

Although UQ-10 is freely soluble in *n*-hexane, it is not extracted from plasma at low temperatures by this solvent. The present work revealed that, when SDS was added to plasma, UQ-10 was extractable. It may be that this compound forms complexes with weakly binding sites on one or more plasma

proteins but that these complexes dissociate at higher temperatures. SDS would dissociate the complexes even at low temperatures by unfolding the proteins and disrupting the binding sites.

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