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Quantification of residual dimethyl sulfoxide in supernatants of haematopoietic stem cells by capillary zone electrophoresis

Pascal Houzé[a,*], Liliane Dal Cortivo[b], Michel Anselme[c], Bernard Bousquet[a], Bernard Gourmel[a]

[a]*Laboratoire de Biochimie, Hôpital Saint Louis, 1 Avenue Claude Vellefaux, 75010 Paris, France*
[b]*Centre de Secteur d'Hémobiologie et de Transfusion Sanguine, Hôpital Saint Louis, 1 Avenue Claude Vellefaux, 75010 Paris, France*
[c]*Société Beckman, 52–54 Avenue des Bourdons, 93220 Gagny, France*

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Abstract

Dimethyl sulfoxide (DMSO) is a chemical compound that is used to preserve haematopoietic stem cells during freezing at −180°C. As DMSO is largely removed by washing before reinjection of cells into a patient, accidents (notably cardiovascular) are infrequent. The lack of a method for evaluating the residual quantities of this product led us to develop a technique for assaying DMSO by capillary zone electrophoresis without extraction. This simple, rapid and precise technique was applied to the supernatant of cell pellets of thirteen patients before and after washing. © 1999 Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

After chemotherapy or intensive irradiation, the injection of cryopreserved haematopoietic stem cells is a method of choice in the treatment of certain cancers, particularly leukemia, lymphomas and some solid tumours [1,2]. Cell integrity during freezing at −180°C is preserved by adding protective substances to the medium [3], particularly, dimethyl sulfoxide (DMSO) [3,4], which is generally used at a concentration of 8–10% (v/v) in human albumin or patient's serum [2]. Under these conditions, cells are highly resistant to freezing and show good viability when reinjected [4]. The advantage of DMSO over glycerol is better diffusion through cell membrane and lower toxicity for man [5,6].

Some studies have reported DMSO metabolism in animals or man with isotopic techniques [7] or chromatographic techniques requiring deproteinisation and neutralisation of the sample [8,9]. However, DMSO toxicity in man is still poorly understood and controversial [1,9]. The toxicity may be dependent on the dose and on the route and, especially, on the rate of administration [10]. During reinjection of stem cells, some teams have reported cardiovascular-related toxicities, such as a lowering of arterial blood pressure, dyspnea and cardiac toxicity [11–13]. The main hypothesis to account for these side effects concerns the quantity of DMSO reinjected with the stem cells [14]. To limit the quantity, stem-cell

*Corresponding author.

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pellets are washed in a buffered glucose–sodium chloride solution before being transfused into the patient [15]. A fractional injection of haemopoietic stem cells may also reduce the risks related to DMSO injection [1].

To our knowledge, no attempt has been made to quantify residual DMSO concentrations in cell pellets or to relate these levels to the untoward effects observed or to the quality of cell preservation. This is expected since methods for repeated assays are not easy to implement and results are needed quickly. We propose a capillary zone electrophoresis (CZE) technique that is simple, rapid and precise, allowing DMSO to be assayed in stem-cell supernatant before and after washing. This technique, which requires no preliminary treatment of the sample, was applied to 13 patients before bone-marrow grafting.

2. Experimental

2.1. Reagents

Pure DMSO was kindly provided by the Braun Medical Laboratories (Boulogne, France). Human albumin was obtained from Sigma-Aldrich (Saint Quentin Fallavier, France). All other chemical reagents, such as sodium decahydrate tetrahydroborate and sodium hydroxide, were of analytic quality and supplied by Sigma-Aldrich. Water for injectable preparations (Frésénius France Pharma, Louviers, France) was used with the different reagents.

2.2. Electrophoresis

CZE was performed using the P/ACE 5500 system (Beckman, Gagny, France) equipped with a variable wavelength UV/visible detector. A fused-silica capillary tube was used [total length (L), 47 cm; injector–detector length (l), 40 cm; internal diameter (I.D.), 75 μm]. The part of the capillary ensuring separation was maintained at a constant temperature by immersion in a coolant circulating in the cartridge with a rectangular detection window (800×100 μm).

Direct detection was performed at 214 nm with a bandwidth of 10 nm. The frequency for data acquisition was 16 Hz. Data were collected and analysed with System Gold® (Beckman, Gagny, France).

All measurements were performed in normal polarity (input anode, output cathode). The capillary was thermostated at 25°C. A constant voltage of 30 kV was applied throughout the analysis. Injections of samples were performed in the hydrodynamic modes, with an injection time of 5 s, under a pressure of 20 p.s.i. (1 p.s.i.−6894.76 Pa). Injections were made in duplicate.

The fused-silica capillary was conditioned before each series of analysis by washing it in 0.1 *M* sodium hydroxide (10 min), then in water (10 min) and finally with the electrolyte solution (10 min).

Between each sample in the same series, the capillary was washed in 0.1 *M* sodium hydroxide (4 min) and then in water (1 min) before equilibration for 3 min with the electrolyte solution, pH 9.2. These washings were intended to improve the reproducibility of the electroosmotic flow and, thus, that of migration time.

The electrolyte solution for CZE was composed of a 50-m*M* solution of sodium borate, pH 9.2, filtered through a 0.45-μm membrane (Polylabo, Strasbourg, France) before use. The pH of the solution was checked on a pH-meter (Radiometer, Neuilly-sur-Seine, France).

2.3. Preparation of the range and samples

2.3.1. Preparation of the sample range

A 1% (v/v) stock solution corresponding to 10 000 ppm was prepared extemporaneously in a 4% (p/v) human albumin solution. Each standard was obtained by diluting this stock solution in 50 m*M* sodium borate buffer to produce final concentrations of between 5000 and 50 ppm.

2.3.2. Sample preparation

Cryopreserved cell pellets were rapidly thawed in a water bath at 40°C. Two volumes of washing solution (phosphate buffer, pH 6.8, containing 2 g/l of glucose and 9 g/l of sodium chloride; Braun Medica, Boulogne, France) was then added to one volume of cells. The cells were then centrifuged on a Cobe-IBM washer for 6 min at 450 *g*. The supernatant was removed and the cell pellet was resuspended in 100 ml of 4% (p/v) human albumin.

DMSO was assayed first in the supernatant of cryopreserved haematopoietic stem cells immediately

after thawing (supernatant before washing) and then after resuspension of the cells in human albumin (supernatant after washing).

No extraction procedure was necessary to perform the assay. Prior to injection, samples were diluted in 50 m*M* sodium borate buffer at 1:100 for supernatants before washing and 1:5 after washing.

3. Results and discussion

3.1. Optimisation of the method

The choice of CZE for DMSO quantification was based on the very short analysis time and the possibility of processing a complex sample without using an extraction procedure. In particular, the presence of proteins was not a limiting factor, as in the case of gas chromatography, which requires preliminary deproteinisation. Moreover, silica is an inert substance that resists the action of DMSO much better than the coated surfaces of chromatographic columns.

3.2. Choice of electrolyte

DMSO is a neutral compound that is used ordinarily as a marker of electroosmotic flow. Our decision to work at pH 9.2 with an electrolyte composed of 50 m*M* sodium borate buffer provided conditions in which the surface state of silica was easily reproducible. Moreover, in our technique, proteins migrate slowly with this electrolyte and they do not seem to interfere with the DMSO peak. Under these conditions, verification of Ohm's law showed that there was a linear relation between the voltage applied and the intensity of the current recorded. It was thus decided to work at the highest voltage possible with the instrument used (30 kV) to ensure a shorter analysis time. Current strength was 70 μA and the mean DMSO migration time was 1.68 min (apparent electrophoretic mobility, 0.00094176), with 154 800 plates per meter of capillary.

3.3. Influence of the dilution medium on the sample

Solutions of DMSO (50 000 ppm) were obtained in a mixture of 4% human albumin and 50 m*M* sodium borate buffer by varying the percentage of the electrolyte (0, 25, 50 and 100%). No significant variation in migration time was noted, but there was a very significant improvement in peak width at mid-height, with a five-fold increase in the number of plates between 0 and 100% sodium borate (Table 1). Therefore, the samples were diluted to the maximum in order to retain the highest possible percentage of 50 m*M* sodium borate buffer.

3.4. Influence of injection time

In capillary electrophoresis, efficiency is improved by the injection of concentrated solutions over short periods [16]. Accordingly, injection time was varied from 1 to 10 s while lowering the concentration of the DMSO solution from 1 to 0.1% (v/v) to maintain a constant capillary load. Table 2 shows a very significant reduction in the number of plates between the injection of a concentrated solution over 1 s and that of a more dilute solution. It is generally recognized that injection time should last from 2 to 5 s when quantitative measurements are performed,

Table 1
Influence of injection medium composition on the efficiency of the system[a]

50 m*M* sodium borate (%)	Migration time (min)	Corrected area (arbitrary units)	Efficiency (plates/m)
0	1.684	667 190	110 242
25	1.676	668 359	113 332
50	1.689	658 182	124 151
75	1.668	650 361	133 682
100	1.679	629 908	154 810

[a] Electrophoretic conditions: fused-silica capillary (L=47 cm, l=40 cm, I.D.=75 μm); electrolyte, 50 m*M* sodium borate buffer, pH 9.2; voltage applied, 30 kV (70 μA current); temperature, 25°C; injection time, 5 s.

Table 2
Influence of injection time on the efficiency of the system[a]

Injection time (s)	Efficiency (plates/m)
1	216 910
2	195 252
3	173 140
4	156 782
5	154 810
10	132 550

[a] Electrophoretic conditions are the same as in Table 1, except for injection time, which ranged between 1 and 5 s.

whereas injection over 1 s leads to considerable variability [16]. Although the efficiency of our system was maximal for shorter injection times, capillary loading over 5 s was chosen to obtain sufficient sensitivity while maintaining satisfactory efficiency. This need for sensitivity was the only justification for a loss of efficiency [17].

3.5. Validation of the method

The optimised method was validated for routine DMSO assays. The criteria retained for this optimisation were similar to those generally used for high-performance liquid chromatography and now applied to capillary electrophoresis [18]: specificity, reproducibility of migration time and of the corrected peak areas of DMSO, linearity, sensitivity and percentage recovery as well as intra-and inter-day accuracy and precision.

3.6. Specificity

DMSO is a neutral compound that, under our conditions, migrated very rapidly in less than 2 min. The specificity of our method was confirmed by the absence of any significant variation in migration time within the same series of measurements or during several series (Table 1). Moreover, the electropherogram of a 4% human albumin solution or of diluted patient's serum showed no significant peak at the migration time of DMSO (Fig. 1a). Overloading stem-cell supernatant with a known quantity of DMSO produced only a single peak at 1.68 min, but with a corrected area greater than that of the reference electropherogram (Fig. 1b and 1c). Finally, a dilution test (two-fold dilution from 1:100 to 1:6) performed on supernatant before washing showed a linear relationship ($y=67.22x+738.06$), with a correlation coefficient of 0.999. These results are all indicative of the good specificity of our method. Other neutral substances (such as methanol) would have had the same electrophoretic behaviour as DMSO. However, in our sample preparation and using our working conditions, neither methanol nor any other neutral substance was used that could have interfered with the technique.

3.7. Precision

To determine between run and within run precision, injections were made 10 times in succession and in 10 different series for the points 100 and 1000 ppm in DMSO. Table 3 indicates the mean values,

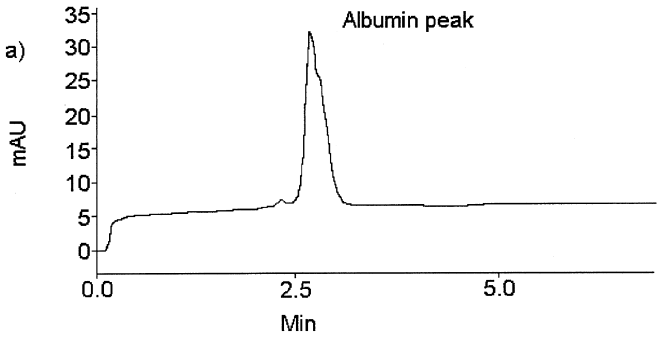

Fig. 1. Specificity study. Electropherograms of a 4% albumin solution (Fig. 1a), of a supernatant before washing, diluted 1:100 in 50 m*M* sodium borate buffer (Fig. 1b) and of the same supernatant overloaded with 100 μl of a 2500 ppm DMSO solution (Fig. 1c). Electrophoretic conditions are the same as in Table 1. Detection was performed at 214 nm with a bandwidth of 10 nm (mAU: milliabsorbance units).

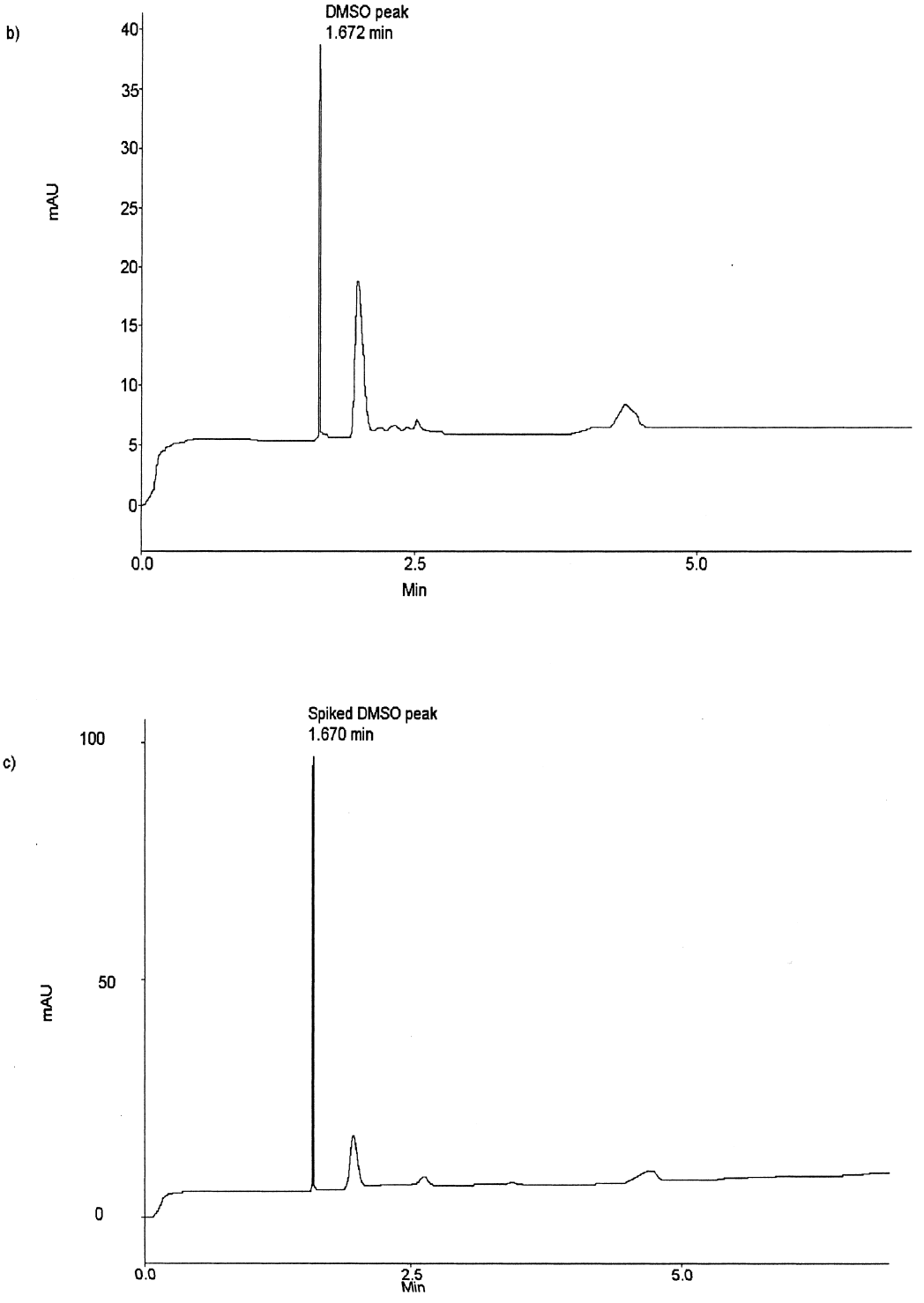

Fig. 1. (*continued*)

Table 3
Precision of the capillary zone electrophoresis technique relative to migration times, corrected areas and concentrations for two DMSO solutions (100 and 1000 ppm)[a]

	DMSO (100 ppm)			DMSO (1000 ppm)		
	Migration time (min)	Corrected area (arbitrary units)	Concentration (ppm)	Migration time (min)	Corrected area (arbitrary units)	Concentration (ppm)
Precision within runs ($n=10$)						
Mean	1.684	7254.6	99.6	1.682	72 947.9	1005.3
R.S.D.	0.008	58.6	1.1	0.006	551.6	8.5
C.V. (%)	0.5	0.8	1.1	0.4	0.8	0.8
Precision between runs ($n=10$)						
Mean	1.683	7129.8	98.9	1.675	72 828.3	998.6
R.S.D.	0.009	176.6	2.25	0.009	1670.1	18.7
C.V. (%)	0.5	2.5	2.3	0.5	2.3	1.9

[a] Electrophoretic conditions are the same as in Table 1.

the relative standard deviations (RSD) and the coefficients of variation (C.V.) for migration times, corrected areas and concentrations. In all cases, precision was excellent, with C.V. less than or equal to 0.8% for reproducibility and 2.5% for repeatability. Good precision was obtained without an internal standard, which is generally used to limit variations due to injection or normalisation of peak areas.

3.8. Linearity

The linearity of the method (peak areas versus concentrations) was evaluated over a concentration range of 50 to 5000 ppm in DMSO. The data for regression analysis performed by the least-squares method gave a mean equation for the curve indicated by $y=67.97x-149.00$. This equation was determined for four calibration ranges obtained on different days. The correlation coefficient ($r>0.999$) confirms the excellent linearity of the method.

3.9. Detection limit (DL) and quantification limit (QL)

The DL, defined as the smallest quanity of DMSO that was clearly distinguishable from baseline, was estimated to be three times the signal-to-noise ratio. The DL was determined by analyses of solutions with decreasing concentrations of DMSO. The DL was estimated to be 5 ppm, which gave a QL of 15 ppm.

3.10. Overload test

This test was performed by overloading a supernatant diluted 1:200 before washing with increasing volumes (10 to 100 μl) of a 2500 ppm DMSO solution in 50 m*M* sodium borate. The percentages of recovery indicated in Table 4 are within the limits of the recovery test (generally between 80 and 120%).

3.11. Application of haematopoietic stem cells to the analysis of supernatants

For 13 patients, the quantities of DMSO contained in stem-cell supernatants were determined before and after washing. DMSO measurement required only preliminary 1:100 or 1:3 dilution of the sample in 50 m*M* sodium borate buffer, pH 9.2. Fig. 2a and b show the typical electrophoretic traces obtained in the same patient before (Fig. 2a) and after (Fig. 2b) washing. For this patient, the stem cells had been cryopreserved in his own serum. It is noteworthy that, after the DMSO peak on the electropherograms, the protein profile is classic from globulins to albumin.

Table 5 shows the DMSO concentrations before and after washing and the percentage of residual DMSO. Except for one case, the concentrations before washing were close to 100 000 ppm or 10% (v/v). Thus, our technique allowed recovery of the quantities of DMSO initially added to the preservation medium. Twelve out of 13 patients had very low

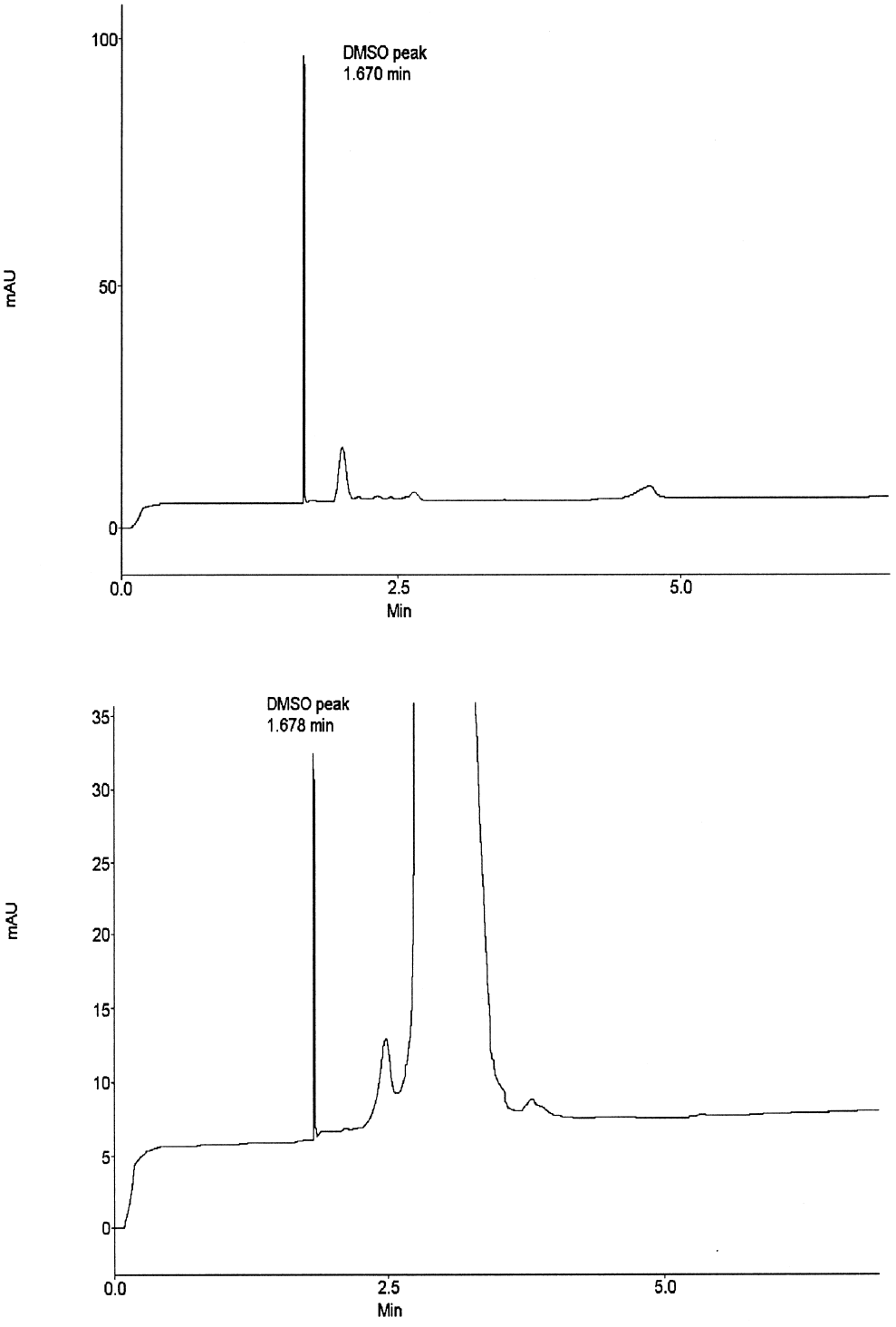

Fig. 2. Typical electropherograms for haematopoietic stem cells before (a) (1:100 dilution in the electrolyte) and after (b) washing (1:3 dilution in the electrolyte). Electrophoretic conditions are the same as in Table 1. Detection was performed at 214 nm with a bandwidth of 10 nm (mAU, milliabsorbance units).

Table 4
Recovery study[a]

DMSO solution (2500 ppm) (μl)	Expected values (ppm)	Measured values (ppm)	Recovery (%)
0	458	462	100
10	498	538	108
20	636	671	105
40	739	779	105
80	1134	1121	99
100	1180	1232	104

[a] Electrophoretic conditions are the same as in Table 1.

Table 5
DMSO concentrations in stem-cell supernatants in 13 patients before and after washing[a]

Patient's name	DMSO before washing (ppm)	DMSO after washing (ppm)	Percentage of residual DMSO
CHJF	136 688	244	0.18
GD	127 265	633	0.50
VB	120 207	1360	1.13
IOF	184 568	863	0.49
BMM	118 021	273	0.23
QM	88 522	272	0.31
MD	87 842	250	0.28
CM	91 903	499	0.54
CC	98 756	356	0.36
MP	100 646	345	0.34
BLJ	100 225	535	0.53
VB	92 907	284	0.31

[a] Electrophoretic conditions are the same as in Table 1.

residual values (between 0.18 and 0.54%). Only subject VB had a residual percentage above one. Therefore, the DMSO assays show that a perfectly standardised technique for washing of cell pellets generally allows the elimination of 99.5 to 99.8% of the quantity of cryopreservative added, which demonstrates the efficiency of the method.

4. Conclusions

The technique described allows for rapid and precise determination of DMSO. This simple method is directly applicable to DMSO assays in complex matrices, such as protein sample, after simple dilution. It is a good method for residual quantification of this cryopreservative before reinjection of stem cells into aplastic subjects. It is directly applicable to the routine assay of residual DMSO, and can rapidly indicate the quality of the product to be reinjected. A more extensive prospective study using this method should allow us to determine the relationships between observed incidents and the quantities of DMSO injected.

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