ELSEVIER

Journal of Chromatography B, 745 (2000) 333–343

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Determination of perfluorodecalin and perfluoro-*N*-methylcyclohexylpiperidine in rat blood by gas chromatography–mass spectrometry

Michel Audran[a], Marie Pierre Krafft[b], Jacques De Ceaurriz[c], Jean-Charles Maturin[c], Marie-Thérèse Sicart[a], Bénédicte Marion[a], Guillaume Bougard[a], Françoise Bressolle[d,*]

[a]*Département de Biophysique, Faculté de Pharmacie, Université Montpellier I, Montpellier, France*
[b]*Institut Charles Sadron, CNRS, Strasbourg, France*
[c]*Laboratoire National de Dépistage du Dopage, Chatenay Malabry, France*
[d]*Laboratoire de Pharmacocinétique Clinique, Faculté de Pharmacie, Université Montpellier I, Montpellier, France*

Received 21 December 1999; received in revised form 8 May 2000; accepted 18 May 2000

Abstract

A gas chromatography–mass spectrometry method (SIM mode) was developed for the determination of perfluorodecalin (cis and trans isomers, 50% each) (FDC), and perfluoromethylcyclohexylpiperidine (3 isomers) (FMCP) in rat blood. The chromatographic separation was performed by injection in the split mode using a CP-select 624 CB capillary column. Analysis was performed by electronic impact ionization. The ions m/z 293 and m/z 181 were selected to quantify FDC and FMCP due to their abundance and to their specificity, respectively. The ion m/z 295 was selected to monitor internal standard. Before extraction, blood samples were stored at −30°C for at least 24 h in order to break the emulsion. The sample preparation procedure involved sample clean-up by liquid–liquid extraction. The bis(*F*-butyl)ethene was used as the internal standard. For each perfluorochemical compound multiple peaks were observed. The observed retention times were 1.78 and 1.87 min for FDC, and 2.28, 2.34, 2.48 and 2.56 min for FMCP. For each compound, two calibration curves were used; assays showed good linearity in the range 0.0195–0.78 and 0.78–7.8 mg/ml for FDC, and 0.00975–0.39 and 0.39–3.9 mg/ml for FMCP. Recoveries were 90 and 82% for the two compounds, respectively with a coefficient of variation <8%. Precision ranged from 0.07 to 15.6%, and accuracy was between 89.5 and 111.4%. The limits of quantification were 13 and 9 μg/ml for FDC and FMCP, respectively. This method has been used to determine the pharmacokinetic profile of these two perfluorochemical compounds in blood following administration of 1.3 g of FDC and 0.65 g of FMCP per kg body weight, in emulsion form, in rat.

Keywords: Perfluorodecalin; Perfluoro-*N*-methylcyclohexylpiperidine

1. Introduction

Perfluorocarbon (PFC) emulsions are being actively developed for use as injectable oxygen carriers (blood substitutes) [1,2]. Perfluorocarbons are highly

*Corresponding author. Tel.: +33-4-6754-8095; fax: +33-4-6754-8075.
E-mail address: Fbressolle@aol.com (F. Bressolle).

0378-4347/00/$ – see front matter
PII: S0378-4347(00)00294-2

effective passive gas carriers, in contrast to hemoglobin, which actively binds oxygen to the iron atom of the heme moiety [3]. PFCs display exceptional physical gas dissolving capacities related to the low cohesive forces that prevail in these apolar liquids [1]. The uptake and release of oxygen (as well as nitrogen, carbon dioxide and other gases) by a PFC depend essentially on the partial pressure of the gas [4]. PFCs dissolve 50–60 vol% of oxygen (under 1 atmosphere of oxygen) and close-to-totally release it to tissues [4,5], while whole blood with normal hemoglobin and hematocrit releases only 25% of the oxygen it transports. PFCs exchange gases more rapidly and more completely than red blood cells do because they load and unload gases by simple diffusion.

The first fluorocarbon emulsion developed for oxygen delivery in humans was based on perfluorodecalin (FDC) and perfluorotripropylamine (Fluosol) [6]. A product close to Fluosol in its formulation (except for the use of a heavier perfluoroamine, perfluoro-*N*-methylcyclohexylpiperidine (FMCP), instead of perfluorotripropylamine), Perftoran (Perftoran Co, Pushchino, Russia), has received approval from the Russian health authorities in 1996 for general use as an antihypoxic agent [7]. A perfluorooctyl bromide emulsion is presently in clinical trials as an oxygen carrier during surgery [2,3].

Because of their efficacy in delivering oxygen to tissues, there is a risk that PFCs be diverted from their intended therapeutic applications and be employed for prohibited uses. These compounds are indeed in instance of being officially included on the International Olympic Committee (IOC) list of products that are prohibited for athletes.

Today, some methods are available to quantify PFCs in biological samples [8–12]. Most of them involved gas chromatography with a flame ionization detector [8,12] or an electron capture detector (ECD) [10]. A sensitive gas chromatography-electron-capture negative ion chemical ionization mass spectrometry method is proposed for the detection of a range of perfluorocarbon tracers in atmosphere [13]. However, these published methods did not report assay validation. In a recent paper, a gas chromatography–mass spectrometry (GC–MS) method for the analysis of perfluorooctyl bromide (perflubron) in rat blood and in expired air has been developed and validated [14].

The objective of the present study was to develop and to validate a GC–MS method for quantifying perfluorodecalin (FDC), and perfluoromethylcyclohexylpiperidine (FMCP) in rat blood. An injectable emulsion of both FDC (13% w/v) and FMCP (6.5% w/v) was used for this purpose. The sample preparation involved sample clean-up by liquid–liquid extraction. This method has enhanced precision due to the inclusion of another perfluorochemical compound (bis(*F*-butyl)ethene, F-44E) as an internal standard and to the high selectivity of gas chromatography coupled with mass spectrometry. This method was validated with respect to accuracy, precision, selectivity, and limits of quantification and of detection according to Good Laboratory Practice Guidelines [15–17]. This method was used to assay samples from a preclinical study in rats.

2. Experimental

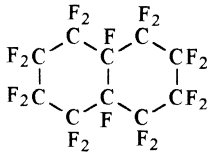
2.1. Materials and reagents

Perfluorodecalin (cis and trans isomers, 50% each) (FDC) (MW 462) came from Air Products (Allentown, PA, USA) and perfluoro-*N*-methylcyclohexylpiperidine (3 isomers) (FMCP) (MW 596) (Fig. 1) was obtained from Perftoran Co. (Pushchino, Russia)

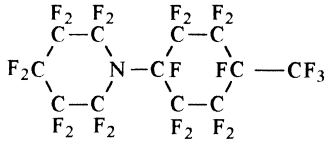
Bis(*F*-butyl)ethene ($C_4F_9CH=CHC_4F_9$, F-44E, MW 464) was a gift from Atochem (Pierre Bénite, France). 1,1,2-trichlorotrifluoroethane (FC 113) was obtained from Fluka (Buchs, Switzerland). Both FDC and FMCP were detoxified [18] and filtered on a 0.22 μm Millipore filter before use.

Synperonic® F-68 (MW ca. 8300), a block copolymer constituted of two terminal polyoxyethylene (POE) blocks flanking a central polyoxypropylene (POP) block, came from Serva (Heidelberg, Germany). Absolute ethanol of HPLC grade was purchased from Carlo Erba (Val de Reuil, France). The 9‰ sodium chloride solution came from Aguettant (Lyon, France).

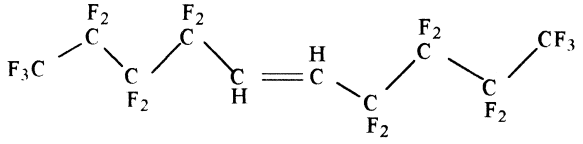
Fifteen dilutions of the FDC/FMCP emulsion, ranging from 0.325 to 130 mg/ml for FDC and 0.162 to 65 mg/ml for FMCP, were prepared in 9‰



Perfluorodecalin (FDC)



Perfluoromethylcyclohexylpiperidine (FMCP)



bis(F-butyl)ethene (F-44E)

Fig. 1. Molecular formula of perfluorodecalin (FDC), perfluoro-*N*-methylcyclohexylpiperidine (FMCP) and bis(*F*-butyl)ethene (F-44E).

aqueous sodium chloride, in a room thermoregulated at 10°C. They were used to establish calibration curves and quality control (QC) samples.

The internal standard F-44E solution was prepared at the concentration of 0.5 ml/l (v/v) in FC 113 and stored at 4°C.

2.2. Fluorocarbon emulsion

2.2.1. Formulation and preparation of the FDC/FMCP emulsion

Physical characteristics of FDC, FMCP and F-44E, and composition of the FDC/FMCP emulsion are given in Tables 1 and 2, respectively.

Table 1
Characteristics FDC, FMCP and F-44E (internal standard)[a]

	FDC	FMCP	F-44E
Chemical formula	$C_{10}F_{18}$	$C_{12}F_{23}N$	$C_{10}F_{18}H_2$
Molecular weight	462	596	464
Boiling point (°C, at 760 mmHg)	142	168	132
Density (g/ml, at 25°C)	1.94	–	1.667
Vapor pressure (mmHg, at 37°C)	12.5	2	12.8

[a] FDC: Perfluorodecalin; FMPC: Perfluoro-*N*-methylcyclohexylpiperidine; F-44E: Bis(*F*-butyl)ethene.

The FDC/FMCP emulsion (200 ml-size batch) was prepared by first dispersing the emulsifier (Synperonic® F-68) in the saline phase with an Ultra-Turrax mixer (Ika-Labortechnik, Staufen, Germany) (8000 rpm) for 5 min at 25°C, then adding the fluorocarbon phase (FDC+FMCP) within 10 min under Ultra-Turrax mixing at 8000 rpm. This coarse premix was mixed vigorously for 15 min at 24000 rpm at 25°C. The final emulsion was obtained by cycling the premix 10 times through a Microfluidizer® (model M-110T, Microfluidics Corp. Newton, MA, USA) at a pressure of 34.5 MPa (345 bars), while the temperature was maintained at 25°C. These operations were all be achieved under oxygen exclusion in a clean room. Immediately after preparation, the emulsion was filtered through a 0.22 μm Millipore membrane in order to eliminate high particles and dusts, then bottled in 20 ml vials (head-space ca. 8%, Teflon lined stopper).

Table 2
Composition of the injectable FDC/FMCP emulsion

Component	% w/v
Perfluorodecalin	13
Perfluoro-*N*-methylcyclohexylpiperidine	6.5
Synperonic® F-68	4.0
NaCl	0.6
KCl	0.039
$MgCl_2$	0.019
$NaHCO_3$	0.065
NaH_2PO_4, H_2O	0.02
Glucose	0.2
Water for injection QSP	

2.2.2. Characterization of the FDC/FMCP emulsion

The emulsion was characterized after preparation, and during aging. The pH and osmolarity of a typical, freshly filtered emulsion batch were measured to be 7.5 and 310 mOsm. Its viscosity was 2.1 cp. The average particle size was 63 nm (±5 nm) after preparation, as measured by photon correlation spectroscopy (Coulter model N4MD Nanosizer, Coulter Electronics, Krefeld, Germany) and by photosedimentation (Horiba Capa 700, Horiba Ltd., Kyoto, Japan). Emulsion aging was monitored by photosedimentation at 25°C.

2.3. Equipment and chromatographic conditions

An HP 5989A GC–MS system (Hewlett-Packard, Palo Alto, CA, USA) was used. The chromatographic separation was performed by injection in the split mode (split: 40 ml/min) of 1 μl of the PFC extract in a CP-select 624 CB capillary column (length: 30 m; I.D. 0.32 mm; film thickness: 1.8 μm) (Chrompack, Middelburg, Netherlands). Helium pressure was 40 KPa. The injector temperature was 200°C and the initial oven temperature was 40°C. After injection, the oven temperature was raised to 75°C at 45°C/min and held for 0.97 min. Then, the column temperature was heated at 60°C/min to 100°C and held for 0.6 min. The transfer line temperature was set to 200°C.

Analysis was performed by electronic impact ionization. The ion source temperature was set to 200°C. The electron energy was 70 eV. FDC and FMPC were analyzed in the SIM mode. Two ions were recorded for each compound: ions m/z 293 $(C_7F_{11})^+$ and m/z 131 $(C_3F_5)^+$ for FDC, and ions m/z 181 $(C_4F_7)^+$ and m/z 131 for FMCP. The ions m/z 293 and m/z 181 were selected to quantify FDC and FMCP due to their abundance and to their specificity, respectively (Fig. 2). The ion m/z 295 $(C_7H_2F_{11})^+$ was selected to monitor F-44E; another ion (m/z 126) was also recorded for the selectivity.

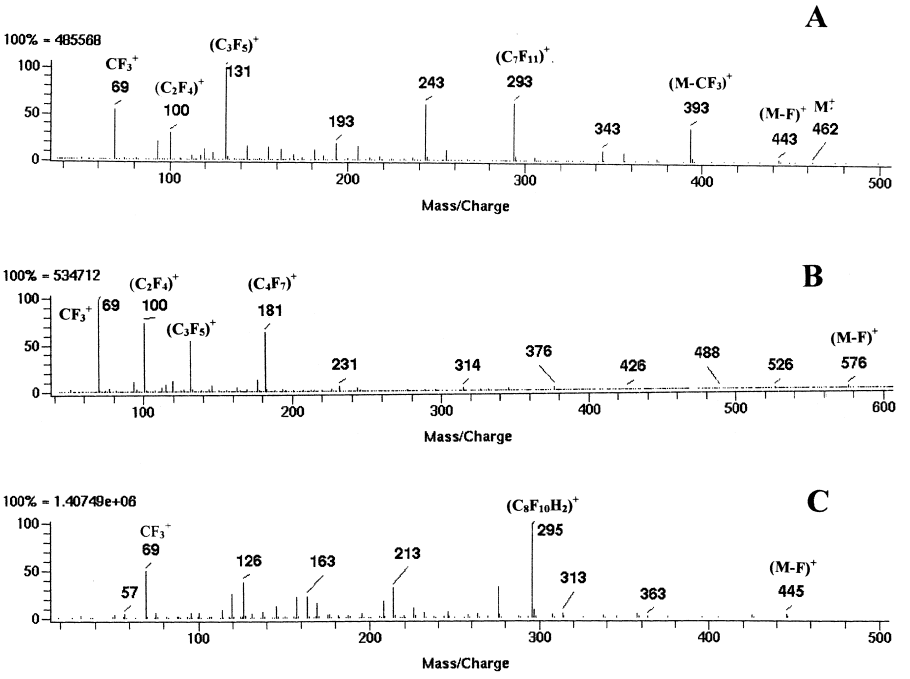


Fig. 2. Mass spectra (SCAN mode) of perfluorodecalin (A), perfluoro-*N*-methylcyclohexylpiperidine (B) and bis(*F*-butyl)ethene used as an internal standard (C).

2.4. Analytical procedures

2.4.1. Calibration curves and quality control samples

All preparations were performed at 10°C. Quantification was based on the internal standard method. Sixty microliters of each diluted FDC/FMCP emulsion were used to spike rat blood samples (1 ml) in order to obtain calibration standards at the concentrations of 0.0195, 0.039, 0.078, 0.117, 0.195, 0.78, 1.17, 1.95, 3.9, 5.85 and 7.8 mg/ml of FDC and 0.00975, 0.0195, 0.039, 0.0585, 0.0975, 0.39, 0.585, 0.975, 1.95, 2.925 and 3.9 mg/ml of FMCP.

QC samples were prepared at the concentrations of 0.026, 0.156, 1.56 and 6.63 mg/ml for FDC and 0.013, 0.078, 0.78 and 3.315 mg/ml for FMCP in rat blood.

Immediately after preparation, a 100-μl aliquot of each blood sample (calibration standards and QC samples) was stored in a 5 ml screw capped glass centrifuge tube at −30°C for at least 24 h before extraction in order to break the emulsion.

The calibration curves and four QC samples were run with every set of ten unknown samples.

2.4.2. Extraction procedure

All experiments were performed at 10°C in order to prevent the losses of FDC and FMCP by evaporation, which occur at room temperature. Prior to analysis, the frozen samples were brought to 10°C and 1 ml of FC 113 (4°C), containing the internal standard, was added; the tubes were immediately sealed. The mixture was vortex-mixed for 60 s, then 100 μl of ethanol (4°C) were added and the tubes were closed. The content was mixed during 15 min in an ultrasonic bath maintained at 4°C, then vortex-mixed for 120 s. The tubes were subsequently centrifuged (4°C) at 1500 g for 5 min and frozen at −30°C for at least 30 min. Just before analysis by GC–MS, the upper ethanolic phase was withdrawn and the lower FC 113 phase put into another 5-ml glass tube; 1 μl of this solution was injected into the chromatograph.

Each injection was performed in duplicate; in the interval between two injections, the extracts were stored at −30°C.

2.5. Data analysis

For both FDC and FMCP, multiple peaks were observed in the chromatogram and therefore the total peak areas of the peaks were used in the calculation of the analyte/internal standard peak area ratio. This latter was then plotted against theoretical concentrations.

Standard calibration curves were obtained from unweighted least-squares linear regression analysis of the data ($y = a + bx$; where x = concentration and y = peak area ratio). Two different calibration curves were built, the first one for low concentrations (0.0195–0.78 mg/ml for FDC and 0.00975–0.39 mg/ml for FMCP) and the second one for high concentrations (0.78–7.8 mg/ml for FDC and 0.39–3.9 mg/ml for FMCP). The quality of fit was evaluated by comparing back-calculated concentrations to the nominal ones.

The resulting slopes and intercepts were used to obtain concentration values for that day's quality control samples and unknown samples.

The linearity of the method was confirmed using classical statistical tests; i.e., comparison of intercept with zero and correlation coefficients with 1. In addition, the normal distribution of the residuals (difference between nominal and back-calculated concentrations) was verified. Moreover, to compare the back-calculated concentrations (C_{TEST}) to the theoretical concentrations (C_{REF}), the Bias (or mean predictor error) was computed as follows:

$$\mathrm{Bias} = \frac{1}{n}\sum_{i=1}^{i=n}[C_{\mathrm{TEST}(i)} - C_{\mathrm{REF}(i)}]$$

2.6. Precision and accuracy

Inter-day and intra-day reproducibilities of the assay were assessed by performing replicate analysis of QC samples in blood against a calibration curve. The procedure was repeated on different days on the same spiked standards to determine inter-day repeatability. Intra-day repeatability was determined by treating spiked samples in replicate the same day.

The accuracy was evaluated as [mean found concentration/theoretical concentration]×100. Precision was given by the coefficients of variation.

2.7. Extraction efficiency

The extraction efficiency (recovery) was determined three times at four concentration levels for FDC (0.026, 0.156, 1.56, and 7.8 mg/ml) and FMCP (0.013, 0.078, 0.78 and 3.9 mg/ml), and at the concentration used during the assay for the internal standard. The peak areas obtained after extraction were compared to those obtained using standard solutions prepared at the same concentrations and injected in the GC–MS system.

2.8. Determination of the limit of quantification (LOQ)

The LOQ estimated on spiked samples was defined as the lowest drug concentration which can be determined with a precision equal or lower than 20% and an accuracy between 100±20% on a day-to-day basis [16,17].

2.9. Specificity

To evaluate the specificity of the method, ten different blank rat blood pools were tested. The retention times of endogenous compounds in the matrix were compared with that of FDC, FMCP and F-44E.

2.10. Stability study

The stability of FDC and FMCP in frozen blood samples (−30°C) was determined by periodic analysis over a three month period. Samples were analyzed after preparation (reference values) and after storage.

2.11. Pharmacokinetic study in rat

The animals used were Wistar strain male rats weighing 250–280 g. The animals, first anesthetized with diethylether, received 1.3 g of FDC and 0.65 g of FMCP per kg body weight in emulsion form into the penis vein. Blood samples (100 μl) were collected from the retro-orbitary sinus in glass tubes coated with sodium heparinate, at the following time: 2, 4, 6, 12, 24, 72 h and 7 days after drug administration.

The determination of FDC and FMCP excreted through expiration was also carried out. Rats having received the FDC/FMCP emulsion were therefore placed in a hermetic glass vessel (9 l-capacity) for 30 min at several intervals of times after drug administration (2, 4 and 6 h, and 1, 2, 7, 11, 15, 21, 28, 32 and 36 days). Five minutes before air collection the atmosphere was homogenized using a ventilator; the air (1 ml) was then sampled through a septum using a gas syringe then injected directly into the gas chromatograph. Concentrations of FDC and FMCP in expired air were determined using calibration curves obtained in the same conditions by evaporating (at 37°C) various proportions of FDC and FMCP in the hermetic vessel; each standard curve contained 5 data points. The regression analyses between peak areas and theoretical concentrations revealed that the method is linear ($r^2 > 0.992$). The equations of regression lines are $y = 1000.3x + 148.4$ for FDC and $y = 12440x + 912.4$ for FMCP where $y =$ concentration and $x =$ peak area. The precision of the method was $\leq 12\%$.

A pharmacokinetic analysis of the blood FDC and FMCP concentration versus time and of the excretion rate of FDC and FMCP through expiration over time (rate plot) was undertaken, using the Pk-fit software [19].

3. Results

3.1. Retention times and specificity

The extraction procedure and the chromatographic conditions allowed clear separation of FDC and FMCP. For each perfluorochemical compound multiple peaks were observed. The observed retention times were 1.78 and 1.87 min for FDC, and 2.28, 2.34, 2.48 and 2.56 min for FMCP. A typical chromatogram derived from a blood sample of a treated animal is shown in Fig. 3B.

Chromatographic analysis of blank blood samples confirmed that there were no endogenous peaks that coeluted with the analytes (Fig. 3A).

3.2. Calibration curves

For calibration curves prepared on different days

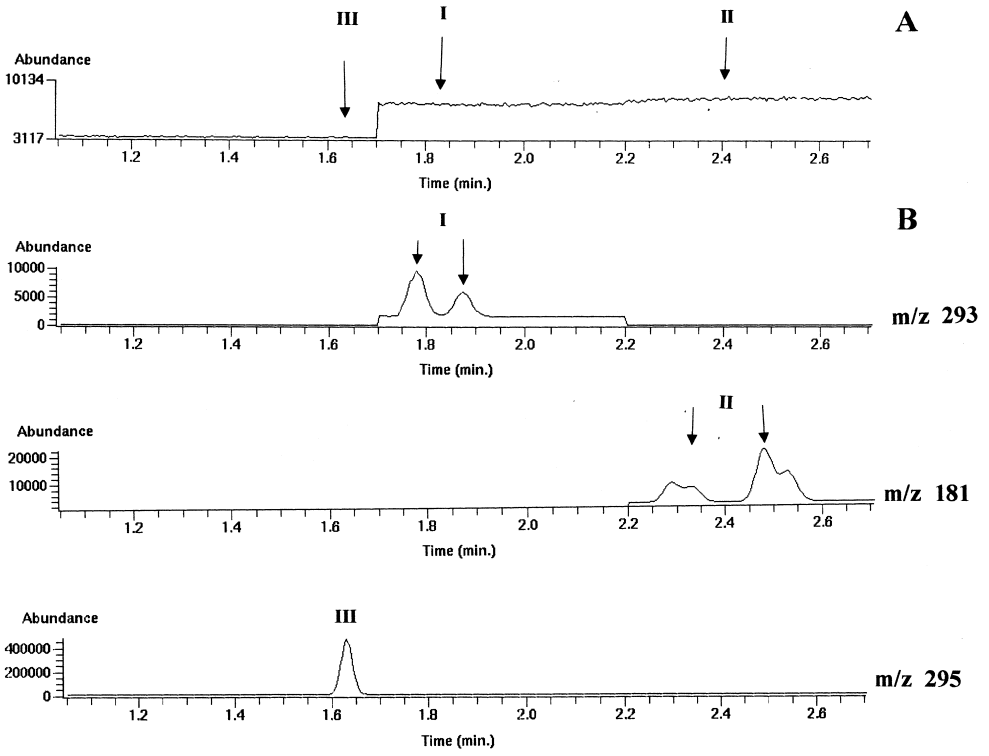


Fig. 3. Typical chromatograms (SIM mode) of drug free rat blood (m/z 293, 181, and 295) (A); and of a blood sample collected 4 h after administration of FDC/FMCP emulsion in a rat (concentrations: FDC, 1.57 mg/ml; FMCP, 1.23 mg/ml) (B). For chromatographic conditions see Sections 2.2 and 2.3.

($n=12$), linear relationships were obtained between the ratio of the area of ions m/z 293 to ion m/z 295 of the internal standard and the concentration of FDC [$y=0.0272$ (±0.0029)$x+0.0002$ (±0.0007) ($r=0.999$, C.V.$=0.14\%$) at low concentrations and $y=0.0265$ (±0.0041)$x-0.0002$ (±0.0044) ($r=0.996$, C.V.$=0.41\%$) at high concentrations]. Likewise linear relationships were found between the ratio of the area of ions m/z 181 to ion m/z 295 and the concentration of FMCP [$y=0.11$ (±0.008)$x+0.0001$ (±0.0007) ($r=0.997$, C.V.$=0.61\%$) at low concentrations and $y=0.115$ (±0.013)$x-0.0047$ (±0.0104) ($r=0.994$, C.V.$=0.65\%$) at high concentrations].

Table 3 reports back-calculated concentrations from the corresponding calibration lines.

The linearity of this method was statistically confirmed. For each PFC studied, a linear regression of the back-calculated concentrations versus the nominal ones provided a unit slope and an intercept equal to 0 (Student's t-test). The distribution of the residuals (difference between nominal and back-calculated concentrations) shows random variations, the number of positive and negative values being approximately equal. Moreover, they were normally distributed and centered around zero. The bias were not statistically different from zero (Student's t-test) (0.0009 and -1.56×10^{-5} for FDC and FMCP, respectively) and the 95% confidence intervals included the zero value (-0.0147–0.0165, -0.0117–0.0116, respectively).

3.3. Extraction efficiency, precision and accuracy

Extraction efficiencies of FDC and FMCP were 90 ± 7 and $82\pm6\%$ ($n=12$), over the range investigated, respectively. For the internal standard it averaged $93\pm8.0\%$ ($n=6$).

For concentrations of calibration standards the precision around the mean value ranged from 0.07 to 15.6% (Table 3). Inter-day and intra-day precision

Table 3
Inter-day precision and accuracy from calibration curves ($n=12$)[a]

m/z 293 (FDC)				*m/z* 181 (FMCP)			
Theoretical concentration (mg/ml)	Back-calculated concentration (mg/ml)	C.V. (%)	Recovery (%)	Theoretical concentration (mg/ml)	Back-calculated concentration (mg/ml)	C.V. (%)	Recovery (%)
0.0195	0.020	7.93	100.6	0.00975	0.010	15.6	97.8
0.039	0.041	6.75	104.3	0.0195	0.021	6.11	105.3
0.078	0.081	2.89	103.7	0.039	0.038	4.90	98.5
0.117	0.114	5.64	97.6	0.0585	0.058	5.62	99.2
0.195	0.192	3.84	98.4	0.0975	0.098	2.69	100.3
0.78	0.781	0.14	100.2	0.390	0.39	0.07	100.0
1.17	1.17	5.41	100.0	0.585	0.60	5.87	103.3
1.95	1.92	5.55	98.3	0.975	0.968	4.37	99.3
3.9	3.91	4.04	100.1	1.95	1.93	5.26	98.7
5.85	5.88	3.15	100.5	2.925	2.89	4.98	98.8
7.80	7.76	2.58	99.5	3.90	3.96	3.76	101.4

[a] FDC: Perfluorodecalin; FMCP: Perfluoro-*N*-methylcyclohexylpiperidine; n = number of replicates.

and accuracy of the method were assessed by analyzing QC samples prepared in rat blood at different concentrations, in replicate, on the same day and on different days. The results are presented in Table 4.

3.4. Limit of quantification

The limits of quantification were 13 and 9 μg/ml for FDC and FMCP, respectively. At these levels, the analytical error was less than 18% and the accuracy ranged from 90 to 105%.

3.5. Stability

The long-term freezer stability in blood indicated that FDC and FMCP were stable during at least 3 months. Compared to the reference values, no statistical difference appeared.

3.6. Pharmacokinetic study

Fig. 4A and B show blood concentration-versus-time profiles. For the two compounds, the data were consistent with a three-compartment model. These three compartments are likely to be blood, re-

Table 4
Assessment of the accuracy and precision of the method[a]

m/z: 293 (FDC)				*m/z*: 181 (FMCP)			
Theoretical concentration (mg/ml)	Experimental concentration (mg/ml)	C.V. (%)	Recovery (%)	Theoretical concentration (mg/ml)	Experimental concentration (mg/ml)	C.V. (%)	Recovery (%)
Intra-day ($n=6$)							
0.026	0.026	7.6	99.5	0.013	0.013	9.2	99.6
0.156	0.14	1.3	89.5	0.078	0.074	5.7	94.4
1.56	1.58	6.6	101.3	0.78	0.78	6.1	100.0
6.63	7.38	2.5	111.4	3.315	3.66	1.9	110.6
Inter-day ($n=22$)							
0.026	0.026	10.8	100.0	0.013	0.013	10.7	100.0
0.156	0.148	5.2	94.9	0.078	0.78	7.8	100.5
1.56	1.62	6.3	103.9	0.78	0.83	5.3	106.9
6.63	6.53	6.2	98.5	3.315	3.26	7.6	98.3

[a] FDC: Perfluorodecalin; FMCP: Perfluoro-*N*-methylcyclohexylpiperidine; n = number of replicates.

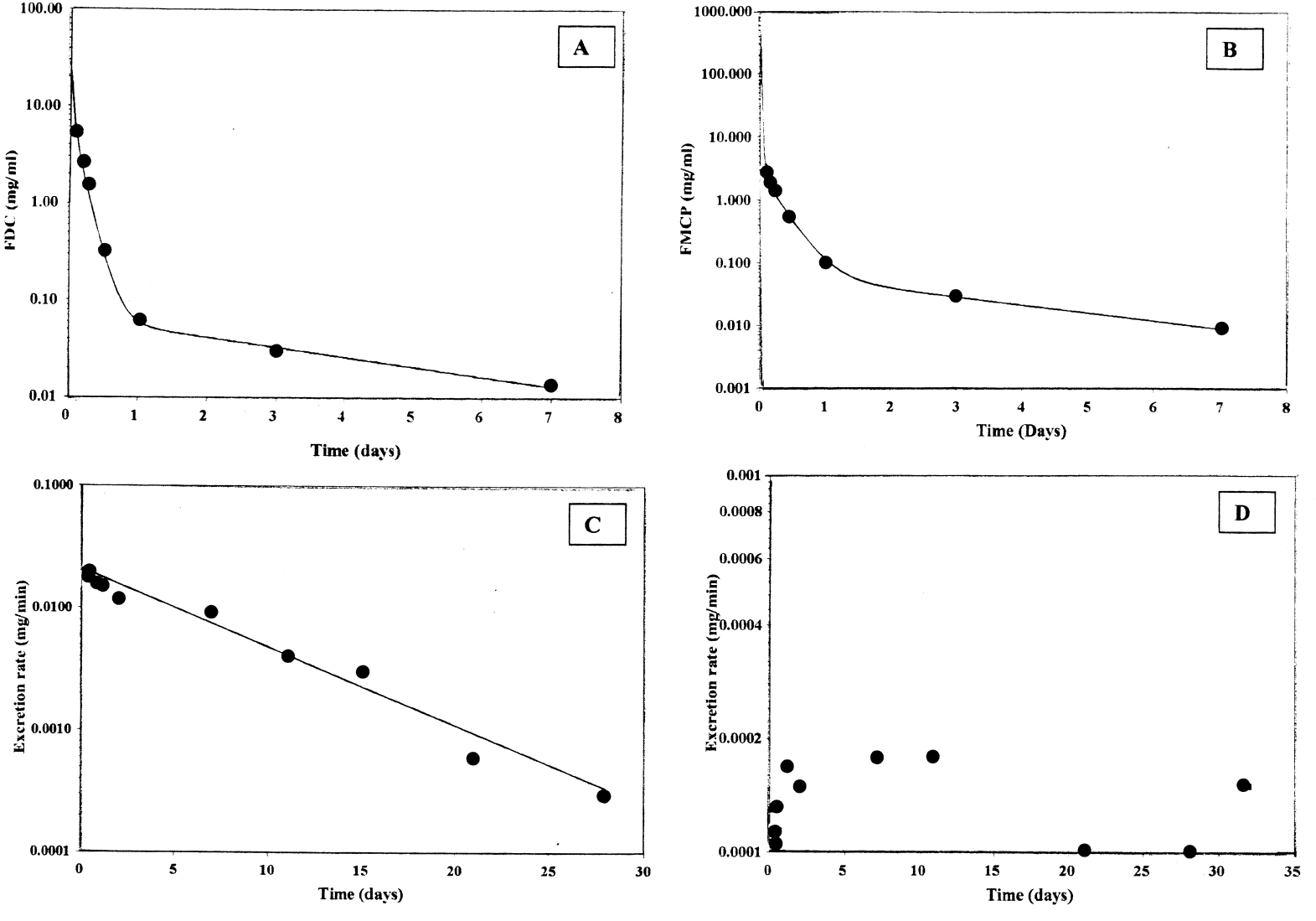


Fig. 4. Kinetics of FDC (A) and FMCP (B) in blood and through expiration (C and D, respectively) after i.v. administration of the FDC/FMCP emulsion in rat.

ticuloendothelial system (RES) organ tissues and adipose tissues. The half-life of the terminal log-linear phase was 4.3 days for FDC, it was 2.6 days for FMCP. The blood clearance was 0.19 and 0.12 ml/min for the two compounds, respectively. Fluorocarbon emulsion droplets are known to be phagocytized by monocytes and macrophages, temporarily stored in the RES organs, primarily the liver, spleen and bone marrow. They diffuse slowly back into the circulation, are taken up by lipid carriers and are delivered to the lungs, from which they are eventually excreted by exhalation with the expired air [1]. In this study, 42 and 1.4% of FDC and FMCP were recovered in the expiratory air after 36 days, respectively. The variations with time of the excretion rate by expiration are presented in Fig. 4C and D. For FDC, an elimination half-life of 5.2 days can be computed; it was of the same order of magnitude as the last observed phase from blood data analysis. The clearance by exhalation was 0.08 ml/min. FMCP is slowly eliminated via expiration, in the terminal phase of elimination, which lasts for at least 36 days, the rate of excretion in expired air fluctuates around a mean of 0.15 μg per min. In view of its molecular weight and absence of lipophilic element, FMCP is predicted to have an organ half-life well above 100 days [1]; a half-life of 90 days has been reported [20].

4. Discussion and conclusions

In this manuscript we describe a GC–MS method that allows quantification of perfluorodecalin (FDC) and perfluoro-*N*-methylcyclohexylpiperidine (FMCP) in rat blood and in the expired air. The extraction procedure is close to that previously published for the analysis of perfluorooctyl bromide (perflubron) [14], but GC–MS conditions have been optimized to detect and quantify specifically these two PFCs in the FDC/FMCP emulsion. Assay performance was assessed both on the basis of the statistical characteristics of individual calibration lines and from the results of quality control samples. The method validation results indicate that the performance characteristics of the method fulfils the requirements for a sufficiently accurate and precise assay method to carry out pharmacokinetic studies.

Due to the high specificity of the GC–MS compared to GC–ECD, the present method could be used to detect misuse by this perfluorocarbon emulsion, a potent oxygen carrier for therapeutic oxygen delivery (blood substitute), by athletes who would intend to artificially increase their performances. The availability of an effective assay for identifying and quantifying the presence of fluorocarbons in blood is of importance for International Olympic Comity and International Union Cyclist, particularly as this method can be used to identify these PFCs in expired air, and should act as a deterrent against such misuse.

References

[1] M.P. Krafft, J.G. Riess, J.G. Weers, The design and engineering of oxygen-delivering fluorocarbon emulsion, in: S. Benita (Ed.), Submicronic Emulsions in Drug Targeting and Delivery, Harwood Academic Publishers, Amsterdam, 1998, pp. 235–333.
[2] J.G. Riess, P.E. Keipert, Update on perfluorocarbon-based oxygen delivery systems, in: E. Tsuchida (Ed.), Present and Future Perspectives of Blood Substitutes, Elsevier, Amsterdam, 1998, pp. 91–102.
[3] J.G. Riess, Fluorocarbon-based oxygen delivery: basic principles and product development, in: T.M.S. Chang (Ed.), Blood Substitutes, Karger Landes Systems, New York, 1998, pp. 101–126.
[4] J.G. Riess, M. Le Blanc, Pure Appl. Chem. 54 (1982) 2383.
[5] R.P. Geyer, Perfluorochemical blood replacement preparations, in: Proceedings of IVth Intl. Symp. Perfluorochemical Blood Substitutes, Excerpta Medica, Amsterdam, 1979, pp. 3–32.
[6] K. Yokoyama, T. Suyama, R. Naito, Development of perfluorochemical (PFC) emulsion as an artificial blood substitute, in: R. Filler, Y. Kobayashi (Eds.), Biomedicinal Aspects of Fluorine Chemistry, Elsevier, Amsterdam, 1982, pp. 191–212.
[7] F.F. Beloyartsev, E.I. Mayevski, B.I. Islamov, Ftorosan-oxygen carrying perfluorochemical plasma substitute, Acad. Sci., Pushchino, USSR, 1983.
[8] K. Yamanouchi, R. Murashima, K. Yokoyama, Chem. Pharm. Bull. 23 (1975) 1363.
[9] P.E. Keipert, S. Otto, S.F. Flaim, J.G. Weers, E.A. Schutt, T.J. Pehura, D.H. Klein, T.L. Yaksh, Artif. Cells Blood Subst. Immob. Biotechnol. 22 (1994) 1169.
[10] C.J. Henry, J.W. Tyler, T. Fields, W.G. Brewer, N. Royer, Artif. Cells Blood Subst. Immob. Biotechnol. 26 (1998) 285.
[11] R.F. Mattrey, A.A. Nemcek, R. Shelton, M.P. André, R.M. Mitten, T. Peterson, Invest. Radiol. 25 (1990) 915.
[12] Iu.D. Aprosin, V.K. Alekseeva, N.I. Afonin, Probl. Gematol. Pereliv. Krovi. 27 (1982) 58.

[13] P. Begley, B. Foulger, P. Simmonds, J. Chromatogr. 445 (1988) 119.
[14] M. Audran, M.P. Krafft, J. De Ceaurriz, J.C. Mathurin, M.T. Sicart, B. Marion, F. Fabre, F. Bressolle, J. Chromatogr. B 734 (1999) 267.
[15] United States Pharmacopoeia XXXIII, The United States Pharmacopeial Convention, Rockville, MD, 1994, p. 1929.
[16] V.P. Shah, K.K. Midha, S. Dighe, I.J. McGilveray, J.P. Skelly, A. Yacobi, T. Layloff, C.T. Viswanathan, C.E. Cook, R.D. McDowall, K.A. Pittman, S. Spector, J. Pharm. Sci. 81 (1992) 309.
[17] F. Bressolle, M. Bromet-Petit, M. Audran, J. Chromatogr. B 686 (1996) 3.
[18] M. Le Blanc, J.G. Riess, D. Poggi, R. Follana, Pharm. Res. 5 (1985) 245.
[19] Pk-fit computer program, version 1.2, 1998, R.D.P.P., Montpellier, France.
[20] S.L. Vorobyev, G.R. Ivanitsky, Pushchino, Russia: Russian Academy of Science, 1994.