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Analysis of [$^2H_7$]methionine, [$^2H_4$]methionine, methionine, [$^2H_4$]homocysteine and homocysteine in plasma by gas chromatography–mass spectrometry to follow the fate of administered [$^2H_7$]methionine

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ABSTRACT

Homocysteine plays a key role in several pathophysiological conditions. To assess the methionine–homocysteine kinetics by stable isotope methodology, we developed a simultaneous quantification method of [$^2H_7$]methionine, [$^2H_4$]methionine, methionine, [$^2H_4$]homocysteine and homocysteine in rat plasma by gas chromatography–mass spectrometry (GC–MS). [^{13}C]Methionine and [^{13}C]homocysteine were used as analytical internal standards to account for losses associated with the extraction, derivatization and chromatography. For labeled and non-labeled homocysteine measurements, disulfide bonds between homocysteine and other thiols or proteins were reduced by dithiothreitol. The reduced homocysteine and methionine species were purified by cation-exchange chromatography and derivatized with isobutyl chlorocarbonate in water–ethanol–pyridine. Quantification was carried out by selected ion monitoring of the molecular-related ions of *N*(*O*,*S*)-isobutyloxycarbonyl ethyl ester derivatives on the chemical ionization mode. The intra- and inter-day precision of the assay was less than 6% for all labeled and non-labeled methionine and homocysteine species. The method is sensitive enough to determine pharmacokinetics of labeled methionine and homocysteine.

1. Introduction

A moderate increase in plasma homocysteine concentration is a risk factor for many pathologic conditions, including cardiovascular disease, congenital abnormalities and neurological disorders [1–5]. However, it remains controversial as to whether the increased risk is mediated directly by homocysteine or whether it may simply be acting as a marker for another metabolite.

Homocysteine is a sulfur non-protein amino acid produced by de-methylation of methionine through the intermediates *S*-adenosylmethionine and *S*-adenosylhomocysteine. Homocysteine is then either re-methylated by accepting methyl group from either betaine or 5-methyltetrahydrofolate to form methionine or catabolized irreversibly to form cystathionine. In the absence of renal impairment, hyperhomocysteinemia is caused either by genetic defects in the enzymes involved in homocysteine metabolism or nutritional deficiencies in vitamin cofactors [6]. To understand the mechanisms of plasma homocysteine elevation, it is necessary to quantitatively assess methionine transmethylation and homocysteine re-methylation and transsulfuration in hyperhomocysteinemic patients.

Stable isotope methodology has provided a useful tool for metabolic and pharmacokinetic investigations for endogenous compounds [7–9]. We previously developed a gas chromatography–mass spectrometry (GC–MS) method for determination of methionine and total homocysteine in plasma with good accuracy and precision by using stable isotopically labeled compounds as analytical internal standards [10]. Stable isotopically labeled methionine has been also used as a biological internal standard to investigate the pharmacokinetic behavior of exogenously administered methionine and the extent of homocysteine re-methylation [11,12]. After administration of [3,3,4,4,*S*-methyl-$^2H_7$]methionine ([$^2H_7$]methionine) to rats, the plasma concentrations of the exogenously administered methionine ([$^2H_7$]methionine), the de-methylated homocysteine ([$^2H_4$]homocysteine), and re-methylated methionine ([$^2H_4$]methionine) simultaneously with endogenous methionine and homocysteine were determined by the double isotope dilution method. The double isotope dilution method, however, required a couple of plasma samples at each time obtained after dosing. A known amount of either labeled or unlabeled compounds (metabolite) was added to only one of the sample as analytical internal standard, and was not added to another sample. Peak-area

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ratios of the two samples were then measured to calculate the concentrations of endogenous and exogenous (labeled) compounds in biological fluids [13–15] (Suppl. 1). The double isotope dilution method is generally time-consuming and often leads to a drawback in accurate quantification of low levels of unlabeled and labeled substances in biological fluids.

The aim of the present study is to develop simultaneous quantification method of [$^2H_7$]methionine, [$^2H_4$]methionine, methionine, [$^2H_4$]homocysteine and homocysteine in plasma by GC–MS using [^{13}C]methionine and [$^{13}C_2$]homocystine as analytical internal standards.

2. Experimental

2.1. Chemicals and reagents

DL-[3,3,4,4-$^2H_4$]Methionine ([$^2H_4$]methionine; >99% atom ^{2}H) and DL-[3,3,3′,3′,4,4,4′,4′-$^2H_8$]homocystine ([$^2H_8$]homocystine; 97.9% atom ^{2}H) were purchased from CDN isotopes (Quebec, Canada). DL-[1-^{13}C]Methionine ([^{13}C]methionine) was purchased from Isotec (Miamisburg, OH, USA). L-[$^2H_7$]Methionine was synthesized in our laboratory as described previously [15]. The isotopic purity and the enantiomeric purity were 99.3% atom ^{2}H and >99.8% enantiomeric excess, respectively. L-Methionine, isobutyl chlorocarbonate and dithiothreitol were purchased from Wako (Osaka, Japan). L-Homocystine was purchased from Nacalai Tesque (Kyoto, Japan). A strong cation-exchange solid-phase extraction column BondElut SCX (H^+ form, size 1 ml/100 mg) was purchased from Varian (Harbor City, OH, USA). All other chemicals and solvents were of analytical-reagent grade and were used without further purification.

2.2. Synthesis of [1,1′-$^{13}C_2$]homocystine

To a solution of [^{13}C]methionine (1.0 g, 6.6 mmol) in liquid ammonia (ca. 100 ml) were added small pieces of metallic lithium (180 mg) at −78 °C. The resulting blue solution was stirred at −78 °C with progress of the reaction being monitored by ^{1}H NMR as follows: aliquots of the solution (ca. 1 ml) were removed, evaporated and re-dissolved in 0.1 M sodium deuteroxide in deuterium oxide, and the disappearance of the *S*-CH_3 proton signal (δ 2.01 ppm) of unreacted DL-[1-^{13}C]methionine was monitored. Additional amounts of lithium were added in two portions (30 mg each) at 2 h intervals. After being stirred for 6 h, ammonium chloride (1 g) was added to the reaction mixture and the color of the solution was discharged. The solution was allowed to stand at room temperature to remove ammonia. The remaining off-white residue was dissolved in water (30 ml) and a continuous stream of oxygen was bubbled into the solution for 1 h. The pH of the solution was adjusted with 1 M HCl to pH 7 followed by standing overnight at 4 °C. The precipitate was collected by filtration, washed with cold H_2O, and dried to obtain [1,1′-$^{13}C_2$]homocystine ([$^{13}C_2$]homocystine) as a colorless solid (0.6 g, 32.8%). Elemental analysis, Calculated for $C_6{}^{13}C_2H_{16}N_2O_4S_2$: C, 35.54; H, 5.97; N, 10.36%. Found: C, 35.54; H, 5.90; N, 10.30%.

2.3. Gas chromatography–mass spectrometry-selected ion monitoring (GC–MS-SIM)

GC–MS-SIM analysis was made with a Shimadzu (Kyoto, Japan) QP2010 quadrupole GC–MS equipped with a data-processing system. A methylsilicone bonded-phase fused-silica capillary column SPB-1 (15 m × 0.25 mm i.d.) with a 0.25 μm film thickness (Supelco, Bellefonte, PA, USA) was connected directly to the ion source. Helium was used as the carrier gas at a column head pressure 100 kPa and total column flow-rate was maintained at 1.0 ml/min. A split–splitless injection system Shimadzu SPL-G9 was operated in the splitless mode with a purge flow-rate of 3 ml/min after 2 min. The initial column temperature was set 120 °C. After the sample injection, it was maintained for 2 min, increased at 30 °C/min to 270 °C and held at 270 °C for 1 min. The temperature of the injector was 250 °C. The mass spectrometer was operated in chemical ionization mode with isobutane as the reactant gas at an electron energy of 70 eV and an emission current of 60 μA. The ion source temperature was 280 °C. SIM was performed on the protonated molecular ions at *m*/*z* 278, 279, 282 and 285 for the *N*(*O*,*S*)-isobutyloxycarbonyl ethyl ester (IBC-OEt) derivatives of methionine, [^{13}C]methionine, [$^2H_4$]methionine and [$^2H_7$]methionine, respectively, and *m*/*z* 364, 365 and 368 for the IBC-OEt derivatives of homocysteine, [^{13}C]homocysteine and [$^2H_4$]homocysteine, respectively.

2.4. Preparation of standards

Stock solutions of methionine (1.53 mg/10 ml), [^{13}C]methionine (3.66 mg/25 ml), [$^2H_4$]methionine (1.57 mg/10 ml), [$^2H_7$]methionine (1.62 mg/10 ml), homocystine (2.63 mg/10 ml), [$^{13}C_2$]homocystine (2.73 mg/10 ml) and [$^2H_8$]homocystine (10.02 mg/10 ml) were prepared in 25 mM hydrochloric acid. The stock solutions were further diluted with 25 mM hydrochloric acid to prepare the standard solutions for calibration curve. Quality control (QC) standard solutions were prepared in the same manner as the calibration standard solutions. Storage of these solutions at 4 °C did not result in any detectable decomposition for more than 3 months.

2.5. Sample preparation for GC–MS-SIM

The protocol was based on an analytical procedure developed by our group [10]. To 50 μl of plasma were added 4.88 nmol of [^{13}C]methionine and 0.51 nmol of [$^{13}C_2$]homocystine as the internal standards dissolved in 0.2 ml of 25 mM hydrochloric acid. Following addition of 0.1 M sodium hydroxide (0.1 ml) and 1% dithiothreitol in acetonitrile (50 μl), the resulting solution was kept at room temperature for 30 min. The sample was deproteinized with 10% trichloroacetic acid (0.2 ml). After centrifugation at 1000 × *g* for 5 min at 4 °C, the supernatant was applied to a BondElut SCX cartridge, which was pre-washed and activated with 3 ml of methanol, 3 ml of a mixture of methanol–0.1 M hydrochloric acid (1:1, v/v) and 3 ml of 0.1 M hydrochloric acid. The cartridge was washed with 1 ml of water and 1 ml of a mixture of water–ethanol (2:1, v/v), and then eluted with 0.5 ml of a mixture of water–ethanol–pyridine (30:16:4, v/v). To the eluent was added isobutyl chlorocarbonate (50 μl) and the solution was mixed on a vortex for 10 s. The sample was extracted with 1 ml of chloroform. After evaporating to dryness under a stream of nitrogen, the residue was dissolved in 20 μl of ethyl acetate. A 0.2–2.0 μl portion of the solution was subjected to GC–MS-SIM.

2.6. Calibration curves and quantification

Each standard solution containing known amounts of methionine (0.51–3.06 nmol/50 μl 25 mM hydrochloric acid), [$^2H_4$]methionine (0.011–5.288 nmol/50 μl 25 mM hydrochloric acid), [$^2H_7$]methionine (0.010–10.102 nmol/50 μl 25 mM hydrochloric acid), homocystine (0.101–0.404 nmol/50 μl 25 mM hydrochloric acid), [$^2H_8$]homocystine (0.005–5.021 nmol/50 μl 25 mM hydrochloric acid) was added to 50 μl portions of rat blank plasma containing endogenous methionine and homocysteine. [^{13}C]methionine (4.88 nmol) and [$^{13}C_2$]homocystine (0.51 nmol) were added to the samples as internal standards. The samples were purified, derivatized and analyzed as described above. After

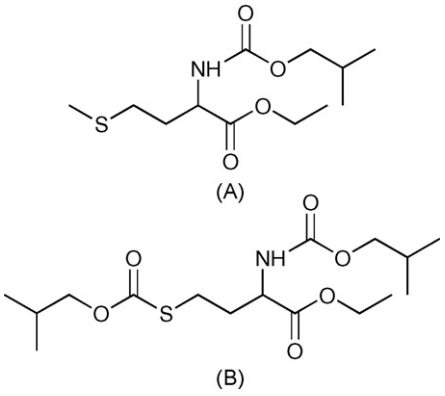

Fig. 1. Structures of *N*(*O*,*S*)-isobutyloxycarbonyl ethyl ester derivatives of methionine (A) and homocysteine (B).

correcting the peak-area values with the values of mutual contributions as shown Table 1, the peak-area ratios (*m*/*z* 278, *m*/*z* 282 and *m*/*z* 285 to *m*/*z* 279 for methionine species and *m*/*z* 364 and *m*/*z* 368 to *m*/*z* 365 for homocysteine species) were determined. The curves were obtained by an unweighted least-squares linear fitting of the peak-area ratios versus the amounts added on each sample. Plasma concentrations of labeled compounds were calculated by comparing the peak-area ratios obtained from the unknown samples with those obtained from the standard mixtures. The concentrations of endogenous methionine and homocysteine were calculated for dividing the *y*-intercept by the slope of the calibration curves.

2.7. Accuracy and precision

QC samples were prepared by spiking the QC solution for methionine (0.51, 1.03, 2.05 nmol/50 μl 25 mM hydrochloric acid), [$^2H_4$]methionine (0.010, 0.103, 1.027 nmol/50 μl 25 mM hydrochloric acid), [$^2H_7$]methionine (0.010, 0.101, 1.010 nmol/50 μl 25 mM hydrochloric acid), homocystine (0.10, 0.20, 0.30 nmol/50 μl 25 mM hydrochloric acid), [$^2H_8$]homocystine (0.005, 0.050, 0.501 nmol/50 μl 25 mM hydrochloric acid) into 50 μl aliquot of rat pooled plasma containing endogenous methionine and homocysteine. Following the addition of [^{13}C]methionine (4.88 nmol) and [$^{13}C_2$]homocystine (0.51 nmol) as internal standards, the samples were subjected to clean-up according to the procedure described above. The samples were analyzed by GC–MS-SIM and the peak-area ratios were measured.

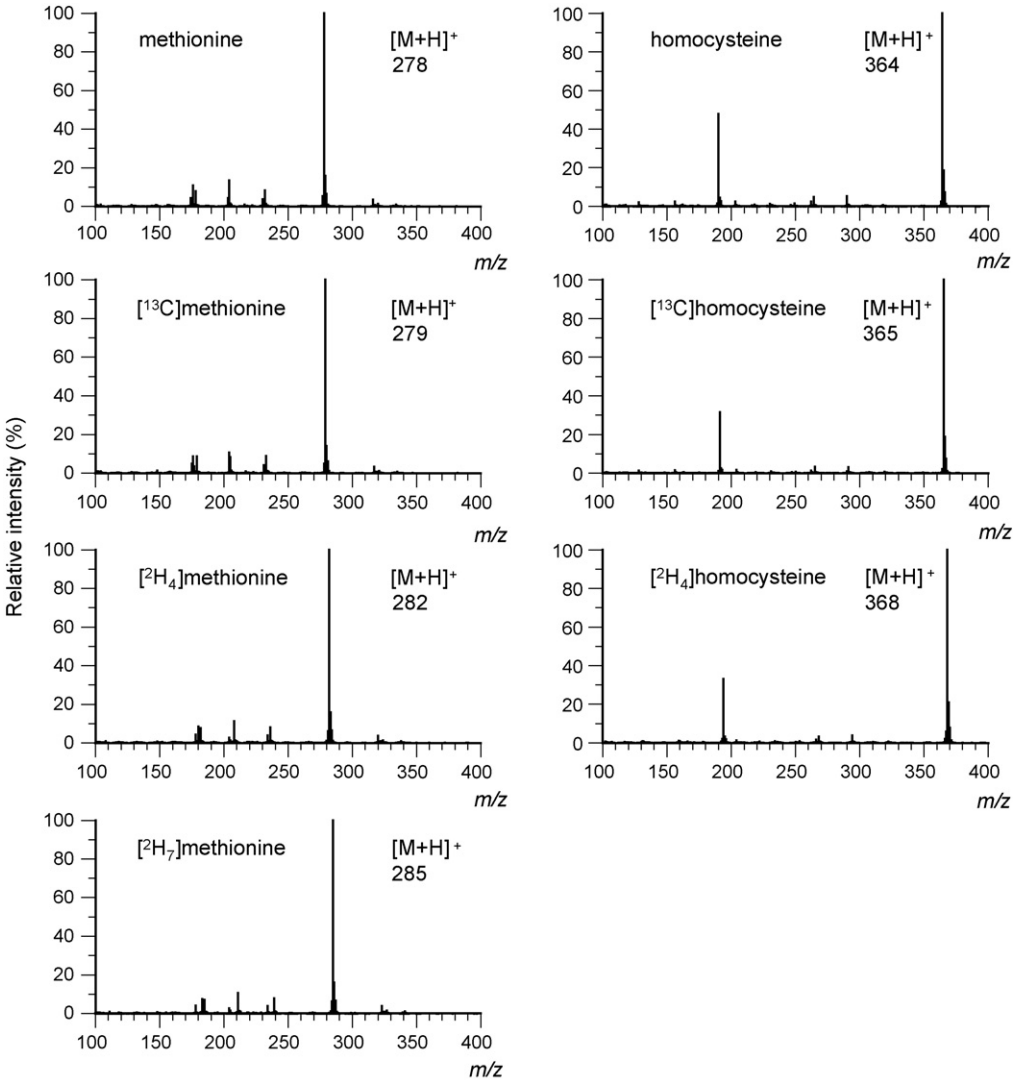

Fig. 2. Chemical ionization mass spectra of *N*(*O*,*S*)-isobutyloxycarbonyl ethyl ester derivatives of methionine, [^{13}C]methionine, [$^2H_4$]methionine, [$^2H_7$]methionine, homocysteine, [^{13}C]homocysteine and [$^2H_4$]homocysteine.

Table 1
Mutual contributions to ion intensity of various species in the channels monitored.

Compound	*m/z* 278	*m/z* 279	*m/z* 282	*m/z* 285
Methionine	100	14.918	0.117	0.023
[^{13}C]Methionine	4.247	100	0.701	0.029
[$^{2}H_4$]Methionine	0.052	0.145	100	0.803
[$^{2}H_7$]Methionine	0.025	0.149	0.961	100

Compound	*m/z* 364	*m/z* 365	*m/z* 368
Homocysteine	100	19.136	0.169
[^{13}C]Homocysteine	2.348	100	1.074
[$^{2}H_4$]Homocysteine	0.055	0.147	100

2.8. Dose experiment

After an overnight fast, male Sprague–Dawley rats ($n = 5$) aged 8 weeks, weighing 240–330 g, were anesthetized with pentobarbital (50 mg/kg body wt., i.p.). Each rat then received into the i.v. bolus injection of [$^{2}H_7$]methionine (30 μmol/kg weight) dissolved in saline (12.5 mg of [$^{2}H_7$]methionine/ml). Heparinized blood samples (150 μl) were obtained from the jugular vein at 5 min before and 0.5, 1, 3, 5, 10, 15, 20, 30, 45, 60, 90, 120, 180, 240 and 300 min after dosing. Plasma was separated and stored at −20 °C until analysis.

3. Results and discussion

One of the unique advantages for use of a stable isotope-labeled compound as a biological internal standard is that an endogenous compound and its exogenous administered labeled analog can be measured separately using GC–MS. The endogenous and exogenous compounds and their metabolites in biological fluids are often analyzed by using the double isotope dilution method. Since the method requires a couple of plasma samples at each time after the administration of labeled compound, it is necessary to collect relatively large volume of blood. Consequently, it is difficult to perform pharmacokinetic studies in small laboratory animals without complications of significant blood loss. This let us to use another type of stable isotopically labeled methionine and homocysteine as analytical internal standards for GC–MS-SIM.

A commercially available [^{13}C]methionine was chosen for an analytical internal standard for determination of methionine, [$^{2}H_4$]methionine and [$^{2}H_7$]methionine. [$^{13}C_2$]Homocystine was prepared from [^{13}C]methionine, and was used for an analytical standard for determination of homocysteine and [$^{2}H_4$]homocysteine.

Homocysteine is a sulfur amino acid with a thiol group that makes it susceptible to oxidation at physiological pH, thereby forming disulfides with other thiols. In plasma, only 1–2% occurs as the reduced form of homocysteine, leaving the remaining 98% in the form of oxidized homocysteine, i.e. in the form of disulfides [16]. Of these, about 75% is bound to protein (mainly albumin), the remainder occurs as non-protein bound disulfides. Moreover, it is known that a redistribution of homocysteine moieties occurs during storage of plasma [17]. To avoid these problems, plasma homocysteine has been measured after reduction of disulfide bonds. The total homocysteine, therefore, is the sum of all forms of homocysteine that exist in plasma [18]. In this study, after the reduction of the disulfide bond with dithiothreitol, the liberated homocysteine and [$^{2}H_4$]homocysteine were determined.

Following deproteinization with trichloroacetic acid and purification by cation-exchange chromatography using a BondElut SCX cartridge, the eluent in a mixture of water–ethanol–pyridine was added to isobutyl chlorocarbonate to form the IBC-OEt derivatives (Fig. 1) as described previously [10]. Fig. 2 shows the chemical ionization (CI) mass spectra of the derivatives of methionine, [^{13}C]methionine, [$^{2}H_4$]methionine, [$^{2}H_7$]methionine, homocysteine, [^{13}C]homocysteine and [$^{2}H_4$]homocysteine. Since IBC-OEt derivatives of methionine, [^{13}C]methionine, [$^{2}H_4$]methionine and [$^{2}H_7$]methionine gave strong protonated molecular ions $[M+H]^+$ at *m/z* 278, 279, 282 and 285, respectively, we have chosen these ions as the monitoring ions. The respective protonated molecular ion at *m/z* 364, 365 and 368 for the IBC-OEt derivatives of homocysteine, [^{13}C]homocysteine and [$^{2}H_4$]homocysteine were also observed in the relatively high intensities and were chosen for quantification by the SIM method.

It was expected that the IBC-OEt derivative of unlabeled methionine would have a significant contribution to the [M+H] + 1 (*m/z* 279) because of the natural abundance of ^{2}H and ^{13}C. In addition, there is also the possibility that the derivative of [^{13}C]methionine could contribute to the [M+H] − 1 (*m/z* 278), [M+H] + 3 (*m/z* 282) and/or [M+H] + 6 (*m/z* 285) peaks. The derivative of [$^{2}H_4$]methionine could contribute to the *m/z* 278, 279 and/or 285 peaks, and the derivative of [$^{2}H_7$]methionine could contribute to the *m/z* 278, 279 and/or 282 peaks. Similarly, the mutual contribution to ion intensities of various species in channels monitored (*m/z* 364, 365, 368) would be also expected for the derivatives of homocysteine, [^{13}C]homocysteine and [$^{2}H_4$]homocysteine. Pure sample of methionine, [^{13}C]methionine, [$^{2}H_4$]methionine, [$^{2}H_7$]methionine, homocysteine, [^{13}C]homocysteine and [$^{2}H_4$]homocysteine were analyzed by GC–MS-SIM and the relative peak intensities are summarized in Table 1. [$^{2}H_4$]Methionine, [$^{2}H_7$]methionine and [$^{2}H_4$]homocysteine possessed sufficiently high isotopic purity and the contributions to the other ions were minor. [^{13}C]Methionine and [^{13}C]homocysteine contain 4.2% and 2.3% of unlabeled analogous, respectively. The contributions of [M+H] + 1 peak to mass spectra of unlabeled methionine and homocysteine are relatively large, 14.9% and 19.1%, respectively. Peak intensities are corrected using the values in Table 1 by the equations described previously [19].

Several different calibration curves were prepared in order to evaluate the amount of internal standards. When a little large amount of [^{13}C] compounds to unlabeled compounds was used, good accuracy and precise were observed. Plasma levels of endogenous methionine and total homocysteine were almost constant (2.93–2.97 nmol/50 μl for methionine, 0.19–0.21 nmol/50 μl for total homocysteine) after a bolus intravenous administration of [$^{2}H_7$]methionine (30 μmol/kg weight) into rats [11]. Therefore, 5 nmol of [^{13}C] methionine and 0.5 nmol of [$^{13}C_2$] homocystine per 50 μl of plasma were chosen for subsequent experiments.

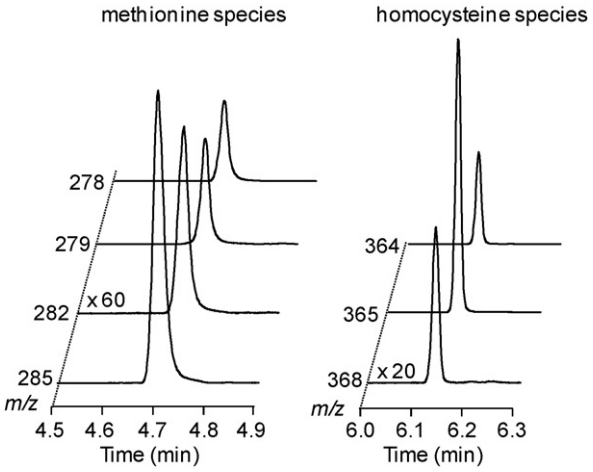

Fig. 3. Representative SIM profiles of rat plasma sample.

Table 2
Intra- and inter-day precision (R.S.D.) and accuracy (R.E.) for the determination of [$^2H_7$]methionine, [$^2H_4$]methionine, methionine, [$^2H_4$]homocysteine and homocysteine spiked to rat plasma.

Spiked (nmol)	Expected (nmol/ml)	Found (mean ± S.D.) (nmol/ml)	R.S.D. (%)	R.E. (%)
Intra-day (n = 3)				
[$^2H_7$]Methionine				
0.01	0.20	0.21 ± 0.01	4.35	5.73
0.10	2.02	2.08 ± 0.04	2.10	3.16
1.01	20.21	19.72 ± 1.36	6.89	−2.41
[$^2H_4$]Methionine				
0.01	0.21	0.20 ± 0.01	3.45	−1.15
0.10	2.05	2.06 ± 0.10	4.67	0.19
1.03	20.54	20.33 ± 0.28	1.36	−1.00
Methionine				
–	–	41.87 ± 0.70	1.67	–
0.51	51.54	51.25 ± 0.14	0.27	−0.56
1.03	61.80	61.07 ± 0.37	0.61	−1.18
2.05	82.34	82.70 ± 0.71	0.86	0.44
[$^2H_4$]Homocysteine				
0.01	0.20	0.20 ± 0.01	3.48	0.58
0.10	2.00	2.00 ± 0.05	2.31	−0.41
1.00	20.03	20.30 ± 0.16	0.81	1.33
Homocysteine				
–	–	7.88 ± 0.05	0.63	–
0.20	11.74	11.63 ± 0.05	0.43	−0.93
0.39	15.66	15.64 ± 0.14	0.90	−0.14
0.59	19.79	19.74 ± 0.20	1.01	0.80
Inter-day (n = 3)				
[$^2H_7$]Methionine				
0.01	0.20	0.20 ± 0.01	4.87	0.51
0.10	2.02	2.10 ± 0.07	3.19	3.84
1.01	20.21	20.52 ± 0.23	1.11	1.57
[$^2H_4$]Methionine				
0.01	0.21	0.21 ± 0.00	1.93	0.87
0.10	2.05	2.07 ± 0.02	0.72	0.99
1.03	20.54	20.43 ± 0.08	0.41	−0.53
Methionine				
–	–	41.88 ± 1.04	2.48	–
0.51	51.54	51.19 ± 0.05	0.10	−0.67
1.03	61.80	61.48 ± 0.35	0.57	−0.53
2.05	82.34	83.11 ± 0.41	0.57	0.94
[$^2H_4$]Homocysteine				
0.01	0.20	0.20 ± 0.01	2.53	−1.09
0.10	2.00	1.99 ± 0.03	1.56	−0.70
1.00	20.03	20.10 ± 0.39	1.94	0.36
Homocysteine				
–	–	7.74 ± 0.15	1.94	–
0.20	11.74	11.59 ± 0.04	0.35	−1.28
0.39	15.66	15.58 ± 0.13	0.83	−0.51
0.59	19.58	19.74 ± 0.19	0.96	0.82

Calibration curves were prepared from a series of samples containing various amounts of methionine, [$^2H_4$] methionine, [$^2H_7$]methionine, homocysteine and [$^2H_4$]homocysteine. When the peak-area ratios were plotted against the amounts added, linearity was excellent over the respective calibration ranges, with corresponding correlation coefficients (R^2) consistently >0.99 for all compounds.

The lower limit of quantification (LOQ) for the present method was determined by spiking 50 μl aliquots rat plasma with [$^2H_7$]methionine, [$^2H_4$]methionine and [$^2H_8$]homocystine. When a relative standard deviation of 10% or greater was used as a criterion for an LOQ [19], the values for [$^2H_7$]methionine, [$^2H_4$]methionine and [$^2H_4$]homocysteine were around 0.085, 0.085 and 0.080 nmol/ml plasma, respectively. The precision and accuracy of the assay were determined by spiking 50 μl-aliquots of blank rat plasma with the QC solutions. The results are presented in Table 2. The estimated amounts were in good agreement with the actual amounts added. The intra- and inter-day precision of the assay was less than 6% for each amino acid at all concentrations. The results demonstrated an excellent reproducibility. In the previously developed double isotope dilution method, the intra-day precisions of 2 nmol/ml plasma were 10.2%, 7.3% and 11.9% for [$^2H_7$]methionine, [$^2H_4$]methionine and [$^2H_4$]homocysteine, respectively (unpublished data). The values of 0.2 nmol/ml plasma were greater than 20% for each amino acid. The present method uses a lower blood sample volume and shows lower variation than the double isotope dilution method.

The present GC–MS-SIM method was applied for the quantification of plasma concentration of [$^2H_7$]methionine, [$^2H_4$]methionine, methionine, total [$^2H_4$]homocysteine and total homocysteine after intravenous administration of [$^2H_7$]methionine (30 μmol/kg weight) to Sprague–Dawley male rats. Representative SIM profiles of plasma samples are shown in Fig. 3. There was no interference from endogenous compounds in the vicinity of the peaks of

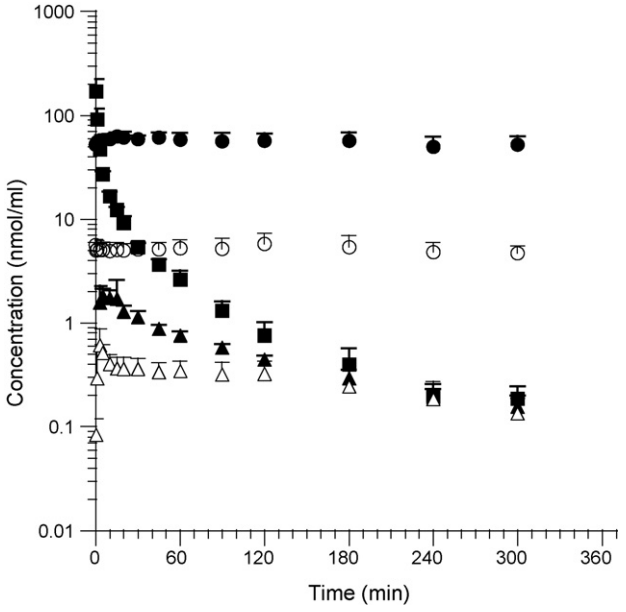

Fig. 4. Semi-logarithmic plots of plasma concentration versus time curves of [$^2H_7$]methionine (■), [$^2H_4$]methionine (▲), methionine (●), total [$^2H_4$]homocysteine (□) and total homocysteine (○) in rats (n=5) after an intravenous administration of [$^2H_7$]methionine (30 μmol/kg weight).

analyses in the SIM. Plasma concentrations of [$^2H_7$]methionine, [$^2H_4$]methionine and total [$^2H_4$]homocysteine with endogenous methionine and total homocysteine could be followed up to 5 h (Fig. 4). Pharmacokinetic studies of [$^2H_7$]methionine or [$^2H_4$]methionine are now in progress and will be described in detail.

4. Conclusions

By using [^{13}C]methionine and [$^{13}C_2$]homocystine as analytical internal standards, the present method provides a sensitive and reliable technique for the simultaneous determination of [$^2H_7$]methionine, [$^2H_4$]methionine and [$^2H_4$]homocysteine with endogenous methionine and homocysteine in plasma with good accuracy and precision. The method can be applied to pharmacokinetic and metabolic studies with a particular interest in the re-methylation of homocysteine to methionine.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jchromb.2009.12.020.

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