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Determination of dichloroacetate and its metabolites in human plasma by gas chromatography–mass spectrometry[1]

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Abstract

Sodium dichloroacetate (DCA) is an investigational drug for the treatment of lactic acidosis, and is also a putative environmental toxicant. We developed and validated a gas chromatography–mass spectrometry (GC–MS) technique that simultaneously measures lactate, DCA and its metabolites, monochloroacetate (MCA), glyoxylate, glycolate and oxalate in human plasma. Following administration of [$^{13}C_{1,2}$]DCA to healthy volunteers, blood samples were collected at various time points and the drug and its metabolites present in plasma were derivatized to their methyl esters by reacting with 12% boron trifluoride–methanol complex. The methyl esters were extracted with methylene chloride and analyzed by GC–MS. The quantitation limits of DCA and metabolites ranged between 0.3 and 1.5 μM. The coefficients of variation of the standards within the entire calibration range were between 0.3 and 14.5%. The bias values ranged between −16.3% and 18.7%. Total recoveries from derivatization and extraction were between 46.9% and 78.5%. The coefficients (r^2) of linear regression of the calibration curves were 0.9882 to 0.9996.

Keywords: Dichloroacetate; Lactate; Monochloroacetate; Glyoxylate; Glycolate; Oxalate

1. Introduction

Sodium dichloroacetate (DCA) is an investigational drug with a potentially broad clinical spectrum, including utility in the treatment of acquired and congenital forms of lactic acidosis [1]. DCA is also a product of water chlorination [2,3], and is a metabolite of certain industrial chemicals [4,5] and drugs, such as chloral hydrate [6] and chloramphenicol [7]. Neurotoxic and hepatotoxic effects of DCA in animals have focused attention on its putative role as an environmental hazard to humans, despite its relatively wide therapeutic index when administered chronically or acutely to children and adults in doses of 12.5 to 50 mg/kg of body weight [1].

Considerable interest exists, therefore, in evaluating the kinetics, metabolism and toxicology of DCA in humans. Accordingly, several analytical techniques have been developed previously to quantitate DCA and its metabolites in biological fluids, with limited success. High-performance liquid chromatog-

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raphy (HPLC), using an ultraviolet-visible (UV–Vis) detection, is applicable only for rather high drug concentrations, because DCA has a low UV absorption [8]. Augmenting the sensitivity of HPLC with a radioactive flow detector can be achieved [9,10], but is impractical for clinical investigations. When DCA concentrations were measured by gas chromatography (GC) using an electron capture detector (ECD), its metabolites glyoxylate, glycolate and oxalate could not be detected [11–14]. Given the selectivity and sensitivity of mass spectrometry (MS), GC–MS methods have been utilized to quantitate DCA, but not its metabolites [15,16]. In this study, we developed and validated a sensitive and selective GC–MS method that is applicable to the study of DCA pharmacokinetics and metabolism in humans. Plasma lactate was measured as a means of correlating the pharmacokinetics with the pharmacodynamics of DCA.

2. Experimental

2.1. Materials

Chemicals used in this investigation were [$^{13}C_{1,2}$]DCA sodium salt (>99.5%, custom synthesized by Cambridge Isotope Laboratories, Inc., Andover, MA, USA), [^{12}C]DCA sodium salt (>99.8%) and oxalic acid dimethyl ester (TCI America, Portland, OR, USA), MCA, glycolic acid, glyoxylic acid monohydrate, 85% (D,L)-lactic acid and methyl esters of DCA, MCA, oxalic acid, (*R*)-(+)-lactic acid, 4-chlorobutyric acid (CBA) and 12% boron trifluoride–methanol complex (Aldrich Chemical Company, Inc., Milwaukee, WI, USA), sodium oxalate (Sigma Chemical Company, St. Louis, MO, USA) and methylene chloride, cyclohexane and *n*-pentane (Fisher Scientific, Pittsburgh, PA, USA). All chemicals were reagent grade or investigational drug grade (DCA) and they were used without further purification. The solvents were pesticide grade. Deionized water was prepared in our laboratory using a Milli-Q Water System (Millipor, Bedford, MA, USA). Fresh frozen human plasma was purchased from Civitan Regional Blood Center (Gainesville, FL, USA).

2.2. Drug administration and plasma sample collection

This study was approved by the Institutional Review Board of the University of Florida and written informed consent was obtained from each of the 20 healthy adult volunteers (10 male) who participated. Prior to the start of the study the subjects underwent three days of a weight maintaining, constant diet at the Clinical Research Center of Shands Hospital, University of Florida. Oxalate rich foods, such as nuts, chocolate and tea, were avoided and only deionized water was drunk or used in food preparation. The same diet was maintained throughout the study, which investigated the kinetics and metabolism of oral and intravenous DCA in a randomized, crossover experimental design. On the day before drug administration, after an overnight fast, 2.5 ml venous whole blood samples were collected in heparinized vacuumed tubes at 0 (8:00 AM), 1/2, 1, 2, 3, 4, 5, 6, 8, 12 and 24 h. Immediately after the 24 hour sample, DCA was administered at a dose of 25 mg/kg orally or by infusion over 15 min in an antecubital vein of the contralateral arm. The dose was repeated every 24 h for five consecutive days. The first and fifth doses contained equal amounts of [^{12}C]DCA and [^{13}C]DCA, while the second, third and fourth doses contained only [^{12}C]DCA. Blood samples were obtained at times 0, 1/12, 1/6, 1/3, 1/2, 1, 2, 3, 4, 5, 6, 8, 12 and 24 h after the first and last doses. Samples were placed on ice and then centrifuged at 2000 rpm/min for 15 min at 4°C in a Sorvall RT6000B refrigerated centrifuge (DuPont, Delaware, NJ, USA). The supernatant plasma fractions were stored at −70°C before deprivatization.

2.3. Derivatization and extraction

The derivatization procedures followed published methods [12,13], with minor modifications. A plasma sample (200 μl) and an internal standard (CBA, 1.75 m*M* in water, 50 μl) were mixed with 500 μl of 12% BF_3-methanol complex in a glass culture tube and the tube was tightly capped with a teflon-lined cap. The mixture was heated at 115°C for 15 min. After cooling, 1 ml of methylene chloride and 1 ml

of water were added to the reaction mixture. The mixture was vortexed using a Vortex Genie 2 mixer (Fisher Scientific, Pittsburgh, PA, USA) for 5 min, allowed to stand for 10 min, and then centrifuged at 3000 rpm in a Beckman J-6B centrifuge (Beckman Instruments, Inc., Palo Alto, CA, USA) at 10°C for 15 min. The methylene chloride layer was transferred to a sample vial for GC–MS analysis.

2.4. GC–MS conditions

The GC–MS system consisted of a Hewlett-Packard (Palo Alto, CA, USA) 5890 series II plus gas chromatograph, a 5972A series mass selective detector, a 6890 series auto-injector, a G1512A autosampler controller and a Vectra multimedia VL2 4/66 computer using ChemStation software (Version C.03.02). The column was an HP-Wax (crosslinked polyethylene glycol) 30 meters long with 0.25 mm I.D. and 0.15 μm film thickness. The carrier gas was helium, purchased from Air Products and Chemicals, Inc. (Allentown, PA, USA), and as used at a flow-rate of 1.21 ml/min (39.1 cm/min) and a head pressure of 8.92 psi on the column. The sample in methylene chloride (1 μl) was injected using a splitless mode. Temperatures of the injection port and mass selective detector interface were set at 150°C and 280°C, respectively. The temperature gradient of the GC oven was programmed to be initiated at 40°C for 4 min, raised to 100°C at 5°C/min, then raised to 240°C at 50°C/min and held at the final temperature for 2 min. An electron impact (EI) ionization mode with the ionization energy of 70 eV was used. Perfluorotributylamine was the calibration compound for the MS detector. The MS detector was calibrated daily before the sample analysis.

Chemical structures of DCA and metabolites were identified by employing a full mass spectra scan mode in comparison with the authentic compounds. The MS detector was calibrated by Standard Spectra Autotune. A selected ion monitoring mode was used for the quantitation of lactate, DCA and its metabolites, and the MS detector was calibrated by Maximum Sensitivity Autotune. As shown in Table 1, specific mass fragments were selected for characterizing methyl esters of MCA, lactate, TCA, DCA, glyoxylate, oxalate and CBA, while only the target ions were used to quantitate these chemicals, using the real time executive integrator of the ChemStation software.

2.5. Calibration curves

Glyoxylate and oxalate are normally present in blood. To overcome interference from these endogenous compounds, [^{12}C]DCA was spiked with 50% [^{13}C]DCA when it was administered. Quantitation of DCA, MCA, glyoxylate and oxalate was based on their corresponding [^{13}C) isotopes. Calibration of lactate, DCA and its metabolites was performed by adding the compounds to water or plasma. The

Table 1
Mass fragments for selected ion monitoring in GC–MS analysis

Compound:	MCA-Me		Lact-Me	DCA-Me		Glyco-Me		Glyox-(Me)$_3$		Oxa-(Me)$_2$		CBA-Me
RT (min):	8.47		9.51	10.76		11.10		11.27		11.70		12.42
Isotope:	[^{12}C]	[^{13}C]	[^{12}C]	[^{12}C]	[^{13}C]	[^{12}C]	[^{13}C]	[^{12}C]	[^{13}C]	[^{12}C]	[^{13}C]	[^{12}C]
Fragments	49	50	33	59[a]	60[a]	45	46	47	48	45	46	49
(m/z)	51	52	42	63	64	59[a]	60[a]	75[a]	76[a]	59[a]	60[a]	59
	59[a]	60[a]	45[a]	77	78	61	62					74[a]
	64	65	59	79	80	90	92					82
	66	67	61	83	84							
	77	78		85	86							
	79	80										
	108	110										
	110	112										

[a]Target ion fragment used for quantitation.

concentration of the internal standard, CBA (438.60 μ*M*),was selected because its peak area was about one half of DCA's peak area at its highest plasma concentration. Peak plasma concentrations following a 25 mg/kg dose were usually less than 1500 μ*M*. Therefore, the concentration range of calibration was set at 0.3–2000 μ*M*. The lowest level was selected to reach the limit of quantitation. Since, under most conditions, each of the DCA metabolites accounted for less than 10% of the parent compound, the concentration ranges of calibration for MCA, glyoxylate and oxalate were set between 0.9–500 μ*M*. Specific concentrations of lactate, DCA and its metabolites tested in water and plasma are listed in Tables 2 and 3, respectively. The calibration curves were plotted as concentration vs. peak area of the compound.

2.6. Recovery of derivatization and extraction

A methylene chloride solution, containing methyl esters (2 m*M* each) of DCA, MCA, L-(+)-lactic acid and oxalic acid, was made as a stock solution. Calibration solutions were made by diluting this stock solution or its corresponding dilutions with methylene chloride to 1.4×10^{-4}, 2×10^{-4}, 8×10^{-4}, 1.4×10^{-3}, 2×10^{-3}, 8×10^{-3}, 1.4×10^{-2}, 2×10^{-2} 8×10^{-2}, 1.4×10^{-1}, 2×10^{-1}, 8×10^{-1} and 1.4 m*M*. Three dilutions at each concentration were made. The calibration curves were obtained by plotting peak area against concentration. Recovery following derivatization and extraction was calculated as the ratio of each concentration of the methyl ester in the methylene chloride extract after derivatization of each compound spiked in water or plasma vs. the concentration of each reference methyl ester added to methylene chloride. The volume of methylene chloride extract vs. the volume of methylene chloride used for extraction was also measured as a factor of recovery. In other words, the recovery was calculated as the ratio of the slope of the calibration curve of each compound obtained from derivatization vs. that of each reference methyl ester directly spiked in methylene chloride and multiplied by a factor of solvent volume change during extraction ($\text{slope}_{(\text{deriv})}/\text{slope}_{(\text{non-deriv})}\times$factor of solvent volume change).

2.7. Method validation

Precision of the method was determined by calculating the intra-day and inter-day coefficients of variance (C.V.%). Intra-day and inter-day variance were measured using the same plasma or water stock solutions prepared for calibration curves. To measure the intra-day variance, five sets of derivatized and extracted samples at each concentration level were generated by using the plasma or water stock solutions. Derivatization and extraction were carried out on each of the following four successive days for measuring the inter-day variance. The intra-day variance was calculated based on the five trial measurements accomplished in the first day, and the inter-day variance was calculated based on the results of five trial analyses carried out on five consecutive days. Accuracy of the method was determined by comparing measured concentrations with those added concentrations, and was expressed as bias (percentage difference between the measured and added concentrations). The intra-day calibration curves were used to quantitate lactate, DCA and its metabolites in human plasma.

3. Results and discussion

3.1. Typical total ion chromatograms (TIC) and internal standard

Presented in Fig. 1 are the TIC of the authentic compounds spiked in water (panel A) and the TIC of a plasma sample of a healthy volunteer collected one hour after intravenous administration of 25 mg/kg DCA ([^{12}C]DCA/[^{13}C]DCA, 50%/50%) (panel B). After investigating a number of halogenated acids, CBA, which did not interfere with analysis of other compounds, was chosen as the internal standard. The concentrations of DCA, [^{13}C]DCA, MCA, glyoxylate, oxalate, lactate and CBA were 124.17, 84.97, 19.88, 24.46, 14.25, 7.69 and 148.19 μ*M*, respectively (panel A). The concentrations of DCA, [^{13}C]DCA, MCA, [^{13}C]MCA, glyoxylate, [^{13}C]glyoxylate, oxalate, [^{13}C]oxalate, lactate and CBA were 270.51, 287.3, 11.35, 11.53, 1.45, 0.21, 5.40, 1.16, 162.71

Table 2
Accuracy and precision of the assay in water

Compound	Intra-day (n=5)			Inter-day (n=5)		
Spiked (μM)	Measured (μM)	C.V. (%)	Bias (%)	Measured (μM)	C.V. (%)	Bias (%)
[^{12}C]DCA	r^2=0.9999			r^2=0.9998		
1986.75	1986.74	3.59	0.00	1982.71	4.14	−0.20
993.38	997.24	7.49	0.39	1008.90	3.31	1.56
496.69	492.58	6.71	−0.83	479.66	7.61	−3.43
248.34	246.54	4.49	−0.72	254.77	5.72	2.59
124.17	126.06	8.71	1.52	127.97	1.98	3.05
31.04	31.12	7.76	0.24	31.17	4.40	0.40
7.76	7.40	9.58	−4.61	7.21	10.79	−7.09
1.94	2.05	3.51	5.54	2.19	17.76	13.05
[^{13}C]DCA	r^2=0.9992			r^2=0.9987		
1359.48	1367.78	3.54	0.61	1358.12	3.58	−0.10
679.74	684.59	7.40	0.71	689.08	2.82	1.37
339.87	333.76	6.30	−1.80	324.88	7.99	−4.41
169.93	170.66	4.04	0.42	173.30	5.80	1.98
84.97	85.81	9.18	0.99	87.99	2.07	3.56
21.24	21.29	6.63	0.21	21.28	4.50	0.18
5.31	5.11	7.81	−3.82	5.09	15.28	−4.19
1.33	1.29	2.41	−2.80	1.55	13.22	17.09
MCA	r^2=0.9998			r^2=0.9993		
317.19	316.36	4.20	−0.26	313.55	3.15	−1.15
158.60	160.98	7.67	1.50	162.86	2.56	2.69
79.30	77.12	2.60	−2.74	75.63	6.77	−4.63
39.65	41.47	3.56	4.58	41.68	8.03	5.12
19.88	19.52	6.46	−1.82	19.62	2.46	−1.30
4.97	5.06	16.74	1.87	4.83	9.10	−2.88
Glyoxylate	r^2=0.9972			r^2=0.9917		
391.89	399.51	6.23	1.95	401.61	9.43	2.48
195.95	181.27	8.75	−7.49	170.07	15.21	−13.21
97.97	95.20	13.11	−2.83	112.17	22.60	14.49
49.05	50.70	17.29	3.36	46.75	11.18	−4.69
24.46	21.31	14.06	−12.89	30.05	26.17	22.86
6.11	6.16	11.66	0.78	5.63	18.46	−7.99
Oxalate	r^2=0.9993			r^2=0.9995		
228.36	228.10	9.22	−0.12	230.35	5.24	0.87
114.18	112.54	1.08	−1.44	115.09	2.95	0.80
57.09	57.32	3.25	0.41	57.05	6.70	−0.06
28.58	29.04	7.24	1.60	28.36	14.44	−0.77
14.25	13.26	10.67	−6.97	14.75	7.22	3.52
3.56	3.63	14.00	1.89	3.24	16.77	−9.15
Lactate	r^2=0.9982			r^2=0.9972		
1147.00	1186.02	2.90	3.40	1225.80	11.87	6.87
573.50	558.25	5.23	−2.66	642.95	9.03	12.11
286.75	298.83	8.95	4.21	259.71	5.44	−9.43
143.38	167.42	3.05	16.77	155.19	8.37	8.24
71.69	65.69	11.10	−8.37	66.41	12.25	−7.36
35.84	40.13	14.13	11.96	40.54	17.21	13.09

Table 3
Recovery, accuracy and variance of the assay in plasma ($n=5$ at each concentration)

Compound	Spiked (μM)	Intra-day (μM)	C.V. (%)	Bias (%)	Inter-day (μM)	C.V. (%)	Bias (%)	Practical Recovery[a] mean±S.D. (%)	Absolute Recovery[b] mean±S.D. (%)	P/W[c] mean±S.D. (%)
[^{12}C]DCA		r^2=0.9996			r^2=0.9987			84.64±2.42	77.87±2.23	92.76±2.41
	1457.73	1453.22	5.18	−0.31	1459.78	5.10	0.14			
	1021.51	1027.82	1.99	0.62	1002.17	5.25	−1.89			
	583.29	586.37	0.33	0.53	616.24	10.04	5.65			
	145.77	141.62	0.59	−2.85	130.02	4.32	−10.81			
	58.32	57.88	11.59	−0.76	57.87	4.52	−0.78			
	14.58	14.33	6.56	−1.71	15.39	10.04	5.57			
	5.83	5.32	0.33	−8.69	5.54	6.61	−4.97			
	1.46	1.66	12.79	13.96	1.60	14.52	9.97			
[^{13}C]DCA		r^2=0.9993			r^2=0.9959			85.32±2.18	78.49±2.01	93.27±2.17
	326.97	330.55	5.11	1.10	321.25	5.10	−1.75			
	228.87	228.04	3.82	−0.36	232.18	5.03	1.45			
	130.79	123.91	4.66	−5.26	139.58	10.92	6.72			
	32.70	33.14	6.66	1.33	36.15	5.36	10.54			
	22.89	22.08	3.24	−3.53	22.17	4.06	−3.16			
	13.08	13.30	4.79	1.65	12.81	3.20	−2.10			
	3.27	3.57	4.61	9.02	3.61	3.83	10.47			
	1.31	1.28	12.54	−2.14	1.23	3.48	−5.80			
MCA		r^2=0.9958			r^2=0.9987			75.66±2.23	69.61±2.06	95.19±2.09
	215.88	212.51	7.01	1.65	214.61	4.08	−0.59			
	53.97	55.70	11.07	3.21	59.77	12.41	10.74			
	37.78	42.08	7.63	11.38	37.75	6.42	−0.07			
	21.59	21.22	11.33	−1.72	20.37	13.59	−5.63			
	5.40	5.90	7.53	9.29	5.78	12.51	7.02			
	3.78	3.41	5.34	−9.67	3.92	8.06	3.83			
Glyoxylate		r^2=0.9933			r^2=0.9882			ND[d]	ND[d]	92.18±13.69
	489.20	493.96	11.21	0.97	561.03	6.88	14.68			
	342.44	334.92	11.85	−2.20	342.05	18.39	−0.11			
	195.68	179.66	8.32	−8.18	190.93	16.79	−2.43			
	48.92	50.07	10.21	2.35	45.62	12.54	−6.75			
	34.24	31.92	9.07	−6.77	40.64	9.07	18.70			
	19.57	20.73	6.72	5.90	16.73	20.51	−14.49			
Oxalate		r^2=0.9955			r^2=0.9962			51.00±5.47	46.92±5.04	91.50±9.13
	261.19	267.80	7.12	2.53	267.50	4.57	2.42			
	182.84	172.71	6.87	−5.54	175.70	6.26	−3.90			
	104.48	103.95	3.22	−0.50	99.85	5.40	−4.43			
	26.12	27.06	4.15	3.58	26.46	3.94	1.31			
	18.28	16.42	9.34	−10.19	17.62	4.27	−3.62			
	10.45	11.38	6.71	8.94	10.79	4.78	3.24			
Lactate		r^2=0.9937			r^2=0.9960			68.86±7.89	63.36±7.26	86.53±11.43
	17946.24	18213.21	8.32	1.49	18049.14	10.76	0.57			
	8973.12	8896.34	5.73	−0.86	9070.46	0.52	1.08			
	4486.56	4152.67	7.12	−7.44	3757.68	6.97	−16.25			
	2243.28	2310.56	3.42	3.00	2364.00	8.24	5.38			
	1121.64	1289.75	9.44	14.99	1329.60	11.14	18.54			

[a]Practical recovery: recovery of the compound as the ratio of slope(deriv)/slope(non-deriv).
[b]Absolute recovery: recovery of the compound as the ratio of slope(deriv)/slope(non-deriv)×factor of solvent volume change.
[c]P/W: ratio of absolute recoveries of the compound in plasma vs. the compound in water.
[d]ND: not determined because authentic 2,2-dimethoxy acetic acid methyl ester was not available.

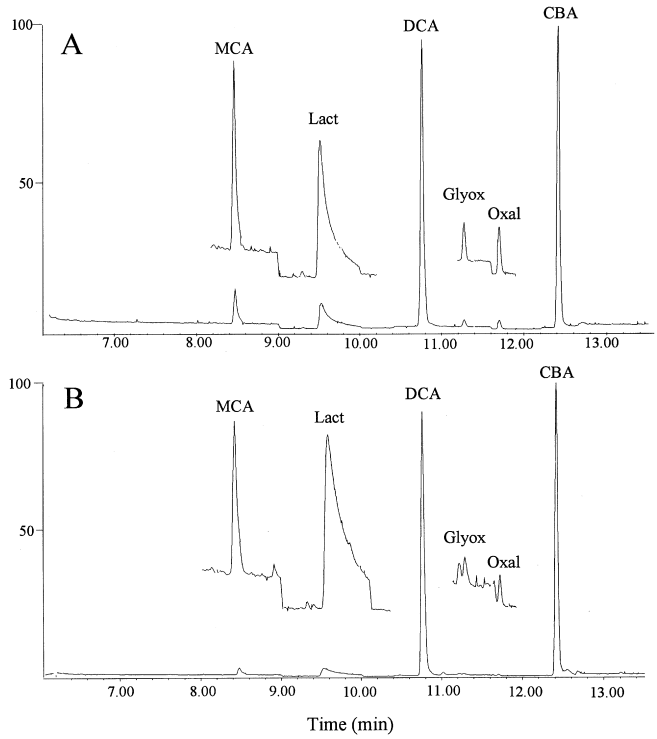

Fig. 1. TIC of GC–MS analysis using a selected ion monitoring mode. Panel A was from the authentic compounds spiked in water, and panel B was from a plasma sample collected from a healthy volunteer one hour after a dose of DCA ([^{12}C]DCA/[^{13}C]DCA 50%/50%, 25 mg/kg, i.v.), where MCA, Lact, DCA, Glyox, Oxal and CBA are methyl esters of MCA, lactate, DCA, glyoxylate, oxalate and CBA, respectively.

and 438.60 μ*M*, respectively (panel B). The retention times of these compounds are presented in Table 1. In addition to the above compounds, glycolate and glycine are also reported to be DCA metabolites [7,8]. We did not detect [^{13}C] labelled glycolate, probably because of its low plasma concentration. When analyzed using an HP–SMS column, we did not find [^{13}C] glycine methyl ester as the DCA metabolite, although the unlabelled compound could be detected (data not presented).

3.2. Accuracy and precision

Quantitation was based on the peak area ratio of the target ions in SIM of the compound and of the internal standard. The calibration curves obtained from intra-day analyses were used to quantitate the plasma samples. Quantitation of DCA and MCA was performed using the calibration curves in plasma, while the naturally occurring compounds, glyoxylate, oxalate and lactate, were determined using the calibration curves in aqueous solutions.

To confirm the accuracy of the present method, some samples were also analyzed by a GC–ECD method [13,14] previously used to determine DCA in human plasma. There was less than 7% difference between the results from our GC–MS method and the GC–ECD technique. The normal human plasma concentration of glyoxylate is reported to be 6–10 μ*M* when analyzed by HPLC [17], and to be less than 4.5 μ*M* when measured by fluorometry [18]. Various methods [19–24] have been used to determine oxalate concentrations in plasma, which are usually reported to be 0.1–6.4 μ*M*. Higher values than this range recorded by some methods may result from conversion of glyoxylate, ascorbic acid or other compounds to oxalate during sample reparation, storage or analysis [19]. In resting, healthy indi-

viduals fasted overnight, the venous plasma lactate concentration is usually about 1 m*M* [25,26]. When the present method was used to analyze 33 samples collected from 11 healthy volunteers in three days, the mean (±S.D.) fasting plasma concentration of unlabelled glyoxylate was 3.05±3.27 μ*M* (range: 0.25–12.78 μ*M*) and the concentration of unlabelled oxalate was 4.55±2.70 μ*M* (range: 0.37–10.95 μ*M*). The basal plasma lactate concentration before DCA administration was 0.85±0.66 m*M* (range: 0.21–2.09 m*M*). These data indicate that the plasma concentrations of glyoxylate, oxalate and lactate measured by the present method are comparable to those determined by previously reported assays.

As shown in Tables 2 and 3, quantitation of the compounds fits well with linear regression (represented by regression coefficient values, r^2) within the measured concentration ranges, while concentrations lower than those listed in Tables 2 and 3 lose their linearities. The r^2 values listed in these tables are the overall regression coefficients within the measured range. Since the plasma concentrations of MCA, glyoxylate and oxalate were lower than 50 μ*M*, the practical calibration curves for these compounds were created between 0.9 to 50 μ*M*. Glyoxylate and its methyl ester may be undergoing partial degradation in solution, in the injector or column, resulting in a high coefficient of variation (C.V.) and bias values. On the other hand, the precision and accuracy of lactate are relatively low, because the peak of methyl lactate was tailing (Fig. 1), which affected the results of peak integration at low levels. However, because the normal plasma concentration of lactate is ~1 m*M*, this tailing is inconsequential for the quantitation of plasma lactate.

3.3. Interference by endogenous compounds

Since the endogenous levels of glyoxylate and oxalate in human plasma are higher than the amounts generated in plasma by DCA metabolism, interference was observed from the natural abundances [^{13}C], especially when the drug metabolite levels were low. This interference was minimized or eliminated by subtracting the values of the control day from the sample being analyzed.

3.4. Recovery of derivatization and extraction

As shown in Table 3, the recovery was obtained as the ratio of the $\text{slope}_{(\text{deriv})}$ of the calibration curve of each compound obtained from derivatization vs. the $\text{slope}_{(\text{non-deriv})}$ of each reference methyl ester directly spiked in methylene chloride, and multiplied by the factor of volume change of methylene chloride during extraction. This factor should be 92%, because it was observed that only 0.92 ml of 1.00 ml methylene chloride used for extraction was recovered. Thus the recoveries of oxalate and lactate were lower than those of the other compounds, probably because their methyl esters are more soluble in the aqueous phase of the extraction mixture, which contained about 28.6% of methanol. The recovery of glyoxylate was not determined, because authentic 2,2-dimethoxy acetic acid methyl ester was not commercially available.

Besides methylene chloride, cyclohexane and *n*-hexane were also tested as the extraction solvents, but their extraction efficiencies were much lower than that of methylene chloride. There were some differences between the recoveries of the compounds spiked in plasma and those spiked in water, as illustrated in Table 3 by the *P*/*W* values (percentage of recovery of the compound spiked in plasma vs. recovery of the compounds spiked in water after derivatization and extraction). These differences might be caused by the higher background in plasma, interference of the endogenous compounds and interaction between the compounds and plasma proteins. For the analysis of samples, no corrections were applied, since internal standards were used for the quantitation of the compounds.

3.5. Limit of quantitation

The limit of quantitation of each compound was determined, based on results obtained from different analyses performed on five consecutive days (Table 4). There were no significant differences between the quantitation limits of DCA and MCA spiked in water and those spiked in plasma. Since the fragment of *m*/*z* 59 used for quantitation of methyl esters of DCA, MCA and oxalate was a common fragment of methyl esters of carboxylic acids, its background was

Table 4
Limit of quantitation of each compound (inter-day, $n=5$)

Compound	In water (μM)	C.V. (%)	In plasma (μM)	C.V. (%)
[^{12}C]DCA	0.49	8.17	0.58	13.75
[^{13}C]DCA	0.33	2.41	0.37	3.38
MCA	1.27	0.67	2.16	5.68
Glyoxylate	1.53	18.10	19.57[a]	6.72
Oxalate	0.89	14.62	10.45[a]	6.71
Lactate	35.84	17.21	560.82[a]	17.05

[a]Serious interference from the endogenous compound in plasma was observed when the concentration was lower.

higher than that of fragment m/z 60 for the methyl ester of [^{13}C]-labelled carboxylic acids. This produced the slight difference between [^{12}C]DCA and [^{13}C]DCA in their limits of quantitation.

Although the limits of quantitation of glyoxylate, oxalate and lactate in plasma were not reached, due to the interference of the endogenous compounds, their limits of quantitation were obtained by spiking the compounds in water. As shown in Table 4, the limits of quantitation of glyoxylate and oxalate in water were comparable with those of DCA and MCA, except for their higher C.V. values. The quantitation limit of lactate was much higher than those of the other compounds, because of the peak tailing of methyl lactate (Fig. 1), which decreased the sensitivity and the precision of this method in detecting lactate. Fortunately, the normal plasma level was about 20 times higher than the limit of quantitation.

3.6. Application

The present method was developed to enhance studies of the metabolism and pharmacokinetics of DCA in humans,and has been applied to more than 1000 plasma samples.In a typical case, a 24-year-old healthy male volunteer received, over 15 min, 25 mg/kg DCA by vein daily for five consecutive days. The first and the fifth doses of DCA contained [^{12}C]DCA and [^{13}C]DCA in a ratio of 50%/50%, while the other doses obtained only [^{12}C]DCA. The plasma samples collected up to 24 h after the first dose of DCA were analyzed using the present GC–MS method, and the plasma concentrations of [^{13}C]DCA and its metabolites, i.e. [^{13}C]-labelled MCA, glyoxylate and oxalate, are shown in (A) and (B) of Fig. 2, respectively. The biological effect of DCA on plasma lactate is shown in Fig. 3, which illustrates both the expected fall in lactate following drug administration and rise in lactate following meals.

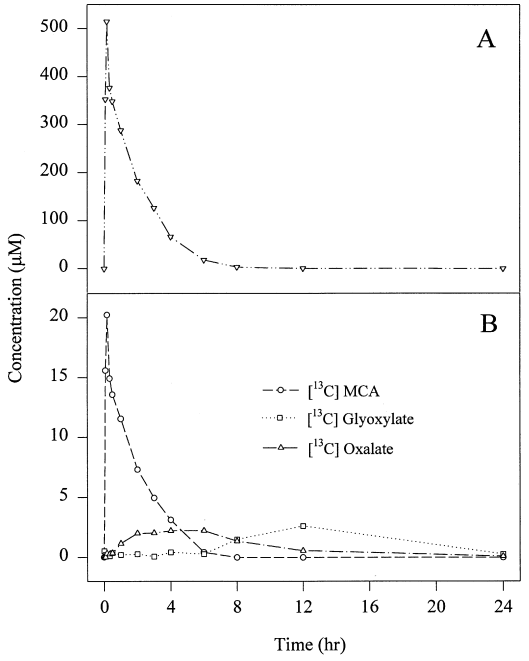

Fig. 2. Plasma concentrations of [^{13}C]-labelled DCA (A) and its metabolites (B), MCA, glyoxylate and oxalate in a normal volunteer after the first dose of DCA ([^{12}C]DCA/[^{13}C]DCA 50%/50%, 25 mg/kg, i.v.).

Although the method described here was principally developed to study DCA biotransformation, it should also prove applicable to the study of the physiology and pathology of glyoxylate and oxalate metabolism. For example, oxalate is an end product of mammalian metabolism and is a common constituent of kidney stones in humans. Calcium oxalate crystals may also be deposited in kidney, blood vessels, myocardium, bone and other tissues, due to acquired or congenital metabolic diseases or to drugs that are metabolized to oxalate [27].

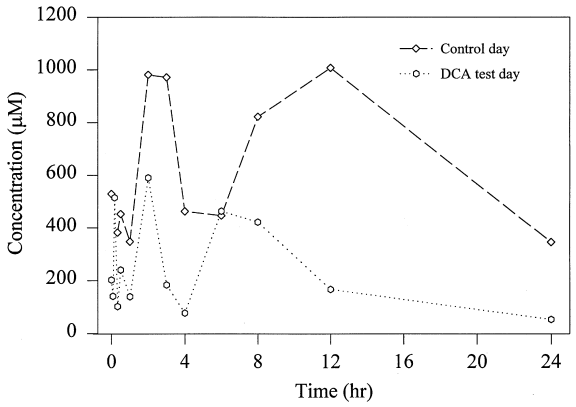

Fig. 3. Plasma concentrations of lactate before and after the first dose DCA in a normal volunteer (25 mg/kg, i.v.). Lactate shows the expected postprandial (meals at 0.5, 3 and 6 h, respectively) increases in plasma concentration.

4. Conclusions

A GC–MS method was successfully developed and validated for simultaneous determination of lactate, DCA and its metabolites MCA, glyoxylate and oxalate in human plasma. Its accuracy and precision are comparable to those other HPLC and GC assays, while its sensitivity and selectivity are superior. In addition, derivatization of samples by our technique is simpler than silylation of organic acids [28], and it can be carried out in an aqueous environment, resulting in a high percentage yield without appreciable degradation or formation of side products.

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References

[1] P.W. Stacpoole, Metabolism 38 (1989) 1124.
[2] A.B. DeAngelo, F.B. Daniel, L. MacMillan, P. Wernsing, R.E. Savage Jr., Toxicol. Appl. Pharmacol. 101 (1989) 285.
[3] H.K. Bhat, M.F. Kanz, G.A. Campbell, G.A. Ansari, Fundam. Appl. Toxicol. 17 (1991) 240.
[4] L.A. Casciola, K.M. Ivanetich, Carcinogenesis 5 (1984) 543.
[5] A.P. Moghaddam, R.R. Abbas, J.W. Fisher, S. Stravrou, J.C. Lipscomb, Biochem. Biophys. Res. Commun. 228 (1996) 639.
[6] R.R. Abbas, C.S. Seckel, J.K. Kidney, J.W. Fisher, Drug Metab. Dispos. 24 (1996) 1340.
[7] L.R. Pohl, G.B. Reddy, G. Krishna, Biochem. Pharmacol. 28 (1979) 2433.
[8] G.N. Henderson, P.O. Whalen, R.A. Darr, S.H. Cu, H. Derendorf, T.G. Baumgartner, P.W. Stacpoole, Drug Devel. Indust. Pharm. 20 (1994) 2425.
[9] E.L.C. Lin, J.K. Mattox, F.B. Daniel, J. Toxicol. Environ. Health 38 (1993) 19.
[10] G. Xu, D.K. Stevens, R.J. Bull, Drug Metab. Dispos. 23 (1995) 1412.
[11] S. Krishna, W. Supanaranond, S. Pukrittayakamee, D. Karter, Y. Supputamongkol, T.M.E. Davis, P.A. Holloway, N.J. White, Metabolism 43 (1994) 974.
[12] R.M. Bersin, C. Wolfe, M. Kwasman, D. Lau, C. Klinski, K. Tanaka, P. Khorrami, G.N. Henderson, T. DeMarco, K. Chatterjee, J. Am. Coll. Cardiol. 23 (1994) 1617.
[13] P. Chu, S.H. Curry, T.G. Baumgartner, G.N. Henderson, P.W. Stacpoole, J. Parent. Sci. Tech. 46 (1992) 16.
[14] J.C. Lipscomb, D.A. Mahle, W.T. Brashear, A. Barton, Drug Metab. Dispos. 23 (1995) 1202.
[15] R.E. Shangraw, R. Winter, J. Hromco, S.T. Robinson, E.J. Gallaher, Anesthesiology 81 (1994) 1127.
[16] W. Dekant, M. Metzler, D. Henschler, Biochem. Pharmacol. 33 (1984) 2021.
[17] X.B. Chen, D.J. Kyle, E.R. Orskov, J. Chromatogr. 617 (1993) 241.
[18] P.M. Zarembski, A. Hodgkinson, Biochem. J. 96 (1965) 218.
[19] J. Costello, D.M. Landwehr, Clin. Chem. 34 (1988) 1540.
[20] M. Petrarulo, E. Cerelli, M. Marangella, D. Cosseddu, C. Vitale, F. Linari, Clin. Chem. 40 (1994) 2030.
[21] M. Petrarulo, E. Cerelli, M. Marangella, F. Maglienti, F. Linari, Clin. Chem. 39 (1993) 537.
[22] M. Petrarulo, O. Bianco, M. Marangella, S. Pellegrino, F. Linari, E. Mentasti, J. Chromatogr. 511 (1990) 223.
[23] L. Hagen, V.R. Walker, R.A. Sutton, Clin. Chem. 39 (1993) 134.
[24] D.M. Wilson, R.R. Liedtke, Clin. Chem. 37 (1991) 1229.
[25] H. Yki-Jarvinen, C. Bogardus, J.E. Foley, Metabolism 39 (1990) 859.
[26] P.W. Stacpoole, Endocrinol. Metab. Clin. North Am. 22 (1993) 221.
[27] R.A.J. Conyers, R. Bals, A.M. Rofe, Clin. Chem. 36 (1990) 1717.
[28] P. Duez, A. Kumps, Y. Mardens, Clin. Chem. 42 (1996) 1609.