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Liquid chromatography–mass spectrometric assay for quantitation of the short-chain fatty acid, 2,2-dimethylbutyrate (NSC 741804), in rat plasma

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Abstract

2,2-Dimethylbutyrate (DMB) is a potential treatment for thalassemia and hemoglobinopathies. To facilitate pharmacokinetic evaluation of DMB, we developed an LC–MS assay and quantitated DMB in plasma of rats after an oral dose of 500 mg/kg. After acetonitrile protein precipitation, DMB and dimethylvaleric acid (DMV) internal standard were derivatized to benzylamides, chromatographed on a Hydro-RP column with acetonitrile, water, and 0.1% formic acid, and detected by electrospray positive-mode ionization mass spectrometry. The assay was accurate (97–107%) and precise (3.4–6.2%) between 100 and 10,000 ng/mL. Recovery from plasma was >62%. Plasma freeze–thaw and room temperature stability were acceptable.

Keywords: Dimethylbutyrate; Metabolism; Mass spectrometry; Validation; Fatty acid; Sickle cell disease; Thalassemia

1. Introduction

β-Thalassemia and sickle cell disease are caused by gene mutations affecting the β-globin chain of adult hemoglobin A and are associated with severe morbidity and early mortality [1,2]. One approach to treating these diseases is reactivating expression of developmentally silenced fetal globin genes, which can functionally substitute for deficient β-globin in β-thalassemia and inhibit sickling in the sickle syndromes [2–7]. Short-chain fatty acids, such as butyric acid, stimulate fetal globin gene expression in experimental models, including patients' cultured cells, transgenic mice, and baboons [8–16]. In clinical trials, short-chain fatty acids have induced fetal hemoglobin and improved total hemoglobin levels, thereby demonstrating proof-of-concept of such an approach to the treatment of hemoglobinopathies [15,17–21]. However, short-chain fatty acids are: (1) rapidly metabolized, necessitating prolonged intravenous infusions or large oral doses and (2) exhibit unwanted anti-proliferative effects on erythroid cells [21]. Development of an orally active, short-chain fatty acid with more favorable pharmacologic properties would be a major advance in the treatment of hemoglobinopathies. 2,2-Dimethylbutyrate (DMB) has such favorable properties; it stimulates fetal globin production, prolongs erythroid cell survival, lacks antiproliferative properties and increases cell proliferation by altering the balance of Bcl-family proteins [14–16]. Because preliminary studies have suggested that DMB has favorable pharmacokinetic properties, such as a longer half-life,

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and an acceptable safety profile [14–16], it has been selected for clinical development. One of the requirements for clinical development is adequate pharmacokinetic characterization of DMB in preclinical models and, eventually, in patients. Such pharmacokinetic studies require a validated analytical method suitable for quantitation of DMB in relevant biological matrices. Because such a method did not exist and because the preclinical toxicology and toxicokinetic studies upon which subsequent clinical trials are being designed were performed in rats, an LC–MS assay was developed to quantitate DMB in rat plasma.

2. Experimental

2.1. Chemicals and reagents

Acetonitrile (Optima grade) was purchased from Fisher Scientific (Fairlawn, NJ, USA). Water was purified using a Q-gard® 1 Gradient Milli-Q system (18.2 MΩ cm, Millipore, Billerica, MA, USA). Formic acid, 2,2-dimethylbutyric acid, dichloromethane, benzylamine, *N*,*N*-diisopropylethylamine, and (bis(2-methoxyethyl)-amino) sulfur trifluoride (Deoxy-Fluor) were purchased from Sigma–Aldrich (St. Louis, MO, USA). 2,2-Dimethylvaleric acid (DMV) was purchased from TCI America (Portland, OR, USA). Control rat plasma was obtained from control animals or purchased from Lampire Biological Laboratories (Pipersville, PA, USA). Nitrogen (99.99% pure) was purchased from Valley National Gases Inc. (Pittsburgh, PA, USA). The sodium salt of DMB (NSC D741804) was supplied by the Developmental Therapeutics Program, National Cancer Institute (Bethesda, MD, USA).

2.2. Preparation of calibration standards and quality control samples

Stock solutions of DMB were prepared at 1 mg/ml in acetonitrile and stored at 4 °C in the dark. On assay days, this solution was serially diluted (in steps of 10-fold) with water to obtain the lower calibration working solutions. These calibration working solutions were diluted in rat plasma to produce 200-μl aliquots of the following DMB concentrations: 100, 300, 1000, 3000, 5000, and 10,000 ng/ml.

Quality control (QC) stock solutions were prepared independently from a separate weighing of DMB and stored at 4 °C in the dark. These solutions were diluted in rat plasma to produce 200 μl aliquots of the following QC samples: QC low (QCL) 200 ng/ml, QC mid (QCM) 1000 ng/ml, and QC high (QCH) 7500 ng/ml. In addition, the QC stock solution was used to prepare rat plasma samples that contained 100 ng/ml DMB, which was the lower limit of quantitation (LLQ) of the assay.

2.3. Sample preparation

Ten microliters of a 10 μg/ml solution of DMV (internal standard) in acetonitrile and 1 ml of acetonitrile were added sequentially to each tube of 200 μl standard, QC, or sample plasma. Samples were vortexed for 1 min at a setting of 10 on a Vortex Genie-2 (Model G-560 Scientific Industries, Bohemia, NY, USA) and then centrifuged at 16,000 × *g* at room temperature for 6 min. The resulting supernatants were transferred to 12 mm × 75 mm borosilicate glass tubes and evaporated to dryness under a stream of nitrogen at 37 °C. Dried residues were re-dissolved in 500 μl of dichloromethane. Following the addition of 10 μl of 4.975% benzylamine in acetonitrile (v/v) and 10 μl of 4.95% *N*,*N*-diisopropylethylamine in acetonitrile (v/v), samples were stored at −20 °C for 20 min. Ten microliters of 60 mg/ml cooled (4 °C) (bis(2-methoxyethyl)-amino) sulfur trifluoride (Deoxy-Fluor) were added, and samples were incubated at −20 °C for an additional 20 min. The reaction mixtures were then evaporated to dryness under nitrogen at 37 °C. Samples were reconstituted in 100 μl of acetonitrile:water (25:75, v/v), transferred to 1.5-ml microcentrifuge tubes, and centrifuged at 16,000 × *g* for 3 min. The resulting supernatants were transferred to autosampler vials, and 5 μl were injected into the LC–MS system.

2.4. Chromatography

The LC system consisted of an Agilent (Palo Alto, CA, USA) 1100 autosampler and binary pump, a Phenomenex (Torrance, CA, USA) Synergi Hydro-RP (4 μm, 2 mm × 100 mm) column kept at ambient temperature, and a gradient mobile phase. Mobile phase solvent A was 0.1% (v/v) formic acid in acetonitrile, and mobile phase solvent B was 0.1% (v/v) formic acid in water. The initial mobile phase composition of 20% solvent A and 80% solvent B was maintained for 4 min at a flow rate of 0.2 ml/min. Between 4 and 13 min, the percentage of solvent A was increased linearly to 50% and was maintained at 50% until 19 min. Between 19 and 20 min, the percentage of solvent A was increased linearly to 100%, and the flow rate was increased to 0.4 ml/min. These conditions were maintained for 2 min. Between 22 and 23 min, the percentage of solvent A was decreased linearly to 20%. Between 23 min and the end of the run at 30 min, the percentage of solvent A was maintained at 20%.

2.5. Mass spectrometry

Mass detection was carried out using a ThermoFinnigan (San Jose, CA, USA) MSQ single quadrupole mass spectrometer with electrospray ionization in positive-ion mode. For both full-scan and single-ion monitoring (SIM) modes, the settings of the mass spectrometer were as follows: capillary voltage 4.0 kV, cone voltage 60 V, and probe temperature 400 °C. In SIM mode, the *m*/*z*-values monitored were 206 and 220 for DMB and DMV, respectively. The dwell time was 0.37 s, and the span was set at 0.2 amu. The LC system and mass spectrometer were controlled by ThermoFinnigan Excalibur software (version 4.0), and data were collected with the same software. The analyte-to-internal standard ratio (response) was calculated for each standard by dividing the area of the analyte peak by the area of the internal standard peak. Standard curves of DMB were constructed by

plotting the analyte-to-internal standard ratio *versus* the known concentration of DMB in each sample. Standard curves were fit by linear regression with weighting by $1/y^2$, followed by the back calculation of concentrations.

2.6. Assay validation procedures

2.6.1. Calibration curve and lower limit of quantitation

DMB added to rat plasma and derivatized as described above was injected into the analytical system at decreasing concentrations to determine the minimal concentration with a signal-to-noise ratio of at least 5:1. Calibration standards and blanks were prepared (see Section 2.2) and analyzed in triplicate to establish the calibration range with acceptable accuracy and precision. Calibration curves were constructed as described above. The deviations of back-calculated concentrations from nominal concentrations, expressed as percentage of the nominal concentration, reflected the assay performance over the concentration range.

2.6.2. Accuracy and precision

The accuracy and precision of the assay were determined by analyzing samples with DMB at the LLQ and QC concentrations in a minimum of 5 replicates in 3 analytical runs together with an independently prepared, triplicate calibration curve. Accuracy (%) was calculated at each test concentration as

$$\frac{\text{mean measured concentration}}{\text{nominal concentration}} \times 100.$$

Assay precision was obtained for each test concentration by using the coefficient of variation of the measured concentration calculated from all 15 determinations for inter-assay precision and calculated from the mean of the three quintuplicate sets for the intra-assay precision.

2.6.3. Selectivity and specificity

To investigate whether endogenous matrix constituents interfered with the assay, four individual batches of control, drug-free rat plasma were processed and analyzed according to the described procedures. Responses of DMB at the LLQ concentration were compared with the response in the blank samples.

2.6.4. Extraction recovery

The recovery of the assay was determined by adding DMB and DMV to the dry residue of 3 blank plasmas that had undergone acetonitrile precipitation and evaporation, and comparing the response to that of samples with DMB and DMV added to 3 fresh plasmas before deproteination and evaporation.

2.6.5. Stability

The stability of a 1 mg/ml DMB stock solution was assessed after storage at 4 °C in the dark for 6 months. The response was compared to a freshly prepared stock solution, and stability was expressed as the percentage recovery of the stored solution relative to the fresh solution. All stability testing in plasma was performed in triplicate at the QCL, QCM, and QCH concentrations. The effect of 3 freeze/thaw cycles on DMB concentrations in plasma was evaluated by assaying samples after freezing and thawing on 3 separate days and comparing them to freshly prepared samples. The stability of DMB in plasma during sample preparation was evaluated by assaying samples before and after 6 h of storage at room temperature. The long-term stability of DMB in plasma was evaluated by assaying samples after 5 months of storage at −80 °C and comparing those results with those of freshly prepared QC samples. To evaluate the stability of DMB in reconstituted samples in the autosampler, we prepared multiple samples of 300 ng/ml DMB in plasma. The samples were extracted, derivatized, evaporated, reconstituted and pooled. The resultant pooled sample was re-injected for 75 runs (37 h).

2.7. Animals and application of the assay

Specific pathogen-free, adult Fischer 344 male rats were purchased from Hilltop Lab Animals (Scottdale, PA, USA). Rats were allowed to acclimate to the University of Pittsburgh Cancer Institute Animal Facility for at least 1 week before being used. To minimize infection, rats were maintained in microisolator cages in a separate room and handled in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) and on a protocol approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh. Ventilation and airflow were set to 12 changes per hour. Room temperatures were regulated at 22 ± 1 °C, and rooms were kept on automatic 12 h light/dark cycles. Rats received Prolab ISOPRO RMH 3000 Irradiated Lab Diet (PMI Nutrition International, St. Louis, MO, USA) and water *ad libitum*, except on the evening before dosing, when all food was removed. Rats were 9–10 weeks old at the time of dosing. Rats were stratified into 5 groups of 3 animals each, and all were fasted overnight prior to dosing. Rats received a single dose of 500 mg/kg DMB sodium salt (equivalent to 414 mg/kg of free DMB) in sterile water (0.005 ml/g body weight) by oral gavage using a 2.5 in., 18-gauge, ball-tipped oral gavage needle and a 3 cm^3 syringe. Heparinized 1 cm^3 syringes fitted with 25-gauge needles were used to obtain blood samples of approximately 400 μl from a lateral tail vein of each of the 3 rats per group at the following specified times after DMB administration. For groups 1, 2, 3, 4 and 5, respectively, blood was collected at before and at 10 min after dosing; at 5 and 45 min after dosing; at 15 and 240 min after dosing; at 30 and 90 min after dosing; and at 60 and 180 min after dosing. Groups of 3 rats were euthanized by CO_2 inhalation at 120, 360, 420, 960 and 1440 min after dosing, and each rat was exsanguinated by collection of blood via cardiac puncture into 10 cm^3 heparinized syringes fitted with 20-gauge needles. Heparinized blood was centrifuged at 13,000 × *g* at room temperature for 4 min to obtain plasma, which was stored at −80 °C until analysis.

Concentration *versus* time data were analyzed noncompartmentally using the Lagrange function [22] as implemented by the LAGRAN computer program [23].

3. Results and discussion

3.1. Method development

The development of an analytical method for DMB presented a number of challenges. DMB is a small, very polar molecule, which made its retention on reverse-phase HPLC columns problematic. We initially evaluated columns, such as the Synergi Hydro-RP 2 mm × 250 mm and Polar-RP 2 mm × 100 mm (Phenomenex, CA, USA) that are designed to retain polar compounds. However, DMB and DMV eluted shortly after the void volume. The use of formic acid, ammonium acetate, and ammonium hydroxide in the mobile phase did not significantly change the retention time of either DMB or DMV. The fact that DMB lacks a chromophore or fluorophore made direct absorbance or fluorescence detection impossible. Mass spectrometric detection was challenging because DMB did not ionize in electrospray positive mode and ionized weakly in electrospray negative mode. Furthermore, the addition of reagents such as formic acid, acetic acid, ammonium acetate, and ammonium hydroxide did not alter the ionization characteristics of DMB. Using the Synergi Hydro-RP column for chromatography and electrospray negative ionization for mass spectrometric detection, detection of 1000 ng/ml of neat compound is allowed. This was not acceptable for our needs and led us to pursue a derivatization strategy to convert DMB and DMV to their respective amides. The derivatization employed involved modifications to a recently published, mild and highly efficient, one-step procedure that used a deoxo-fluoro reagent [24] to convert DMB and DMV to *N*-benzyl-2,2-dimethylbutyramide (BDMB) and 2,2-dimethylpentanoic acid benzylamide (BDMV), respectively (Fig. 1). The original derivatization method was developed for GC–MS with electron impact ionization. Through experimentation, we were able to modify the original method without any loss in efficiency when assessed by LC–MS. The first modification involved replacing the $NaHCO_3$ quenching step and *n*-heptane extraction step, which were included in the original method to remove aqueous material, with direct evaporation of the reaction mixture under a stream of nitrogen at 37 °C. This dried residue was then resuspended in 100 μl of the initial mobile phase, centrifuged, and 5 μl of the resulting supernatant were injected into the LC–MS. This shortened sample processing time and increased sensitivity; however, it did result in a more complex sample matrix. To counter this, we examined the effect of reactant concentration on amide production and found that a 20-fold decease in reactant concentration was tolerated without a decrease in amide production. We also found that 5 μl was a suitable injection volume, as injection of larger volumes led to a clogged sample cone after <24 h of run time.

3.2. Validation of the assay

3.2.1. Mass spectrometry and chromatography

Approximately 50 mg each of DMB and DMV internal standard were converted to their respective benzyl amides, BDMB and BDMV, as described above and purified by flash chromatography on a silica column with ethyl acetate:heptane (20:80, v/v) as the eluting solvent. The purified compounds were determined to be 99% pure by GC–MS, using an Agilent 6890 gas chromatograph fitted with an HP-5 column (30 m × 0.25 mm i.d., 0.25 μm film thickness) and interfaced with an Agilent 5973N mass spectrometer. The structures of BDMB and BDMV were confirmed by ^1H and ^{13}C NMR, using a Varian Mercury 400 spectrometer (Varian Inc., Palo Alto, CA, USA) with spectra recorded at 400 and 100 MHz, respectively. BDMB was diluted in acetonitrile and continuously infused at a rate of 10 μl/min into the HPLC flow, which was an isocratic mobile phase of acetonitrile:water:formic acid (50:50:0.1, v/v/v) pumped at 0.2 ml/min. The tuning parameters of the mass spectrometer were adjusted to maximize the intensity of the $[M+H]^+$ ion. The procedure was repeated for BDMV, and the *m/z* ratios of 206 and 220 were chosen for SIM of BDMB and BDMV, respectively. With the chromatographic conditions described, BDMB and BDMV displayed retention times of approximately 16.9 and 18.5 min, respectively (Fig. 2). There was baseline separation of both compounds. To test for interference, 4 different sources of rat plasma were analyzed. None of the sources tested showed interference with DMB or DMV (data not shown).

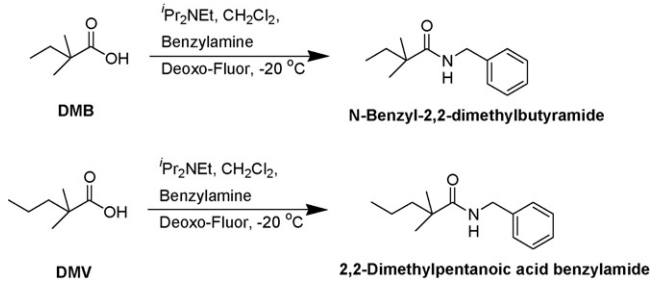

Fig. 1. Reaction of DMB and DMV with benzylamine to form *N*-benzyl-2,2-dimethylbutyramide and 2,2-dimethylpentanoic acid benzylamide.

Table 1
Accuracy and precision of DMB calibration points from three successive triplicate standard curves in rat plasma

	[DMB] (ng/ml)					
	100	300	1000	3000	5000	10,000
Mean	101.0	294.3	1019.7	3113.7	5343.5	9391.6
S.D.	5.40	12.47	45.68	167.28	354.38	700.87
CV[a] (%)	5.35	4.24	4.48	5.37	6.63	7.46

[a] Coefficient of variation.

3.2.2. Linearity

Triplicate standard curves of DMB in plasma were performed on 3 sequential days. The assay proved to be linear and acceptable, as the regression coefficients were >0.99 for each of the three standard curves with $1/y^2$ weighting (data not shown). The lower limit of quantitation was determined to be 100 ng/ml, as the signal-to-noise ratio was >5 at this concentration, and there was acceptable accuracy and precision. The individual values for the mean and standard deviations of the back-calculated values at each nominal DMB concentration used in the standard curves are displayed in Table 1, as is the accuracy calculated from those values.

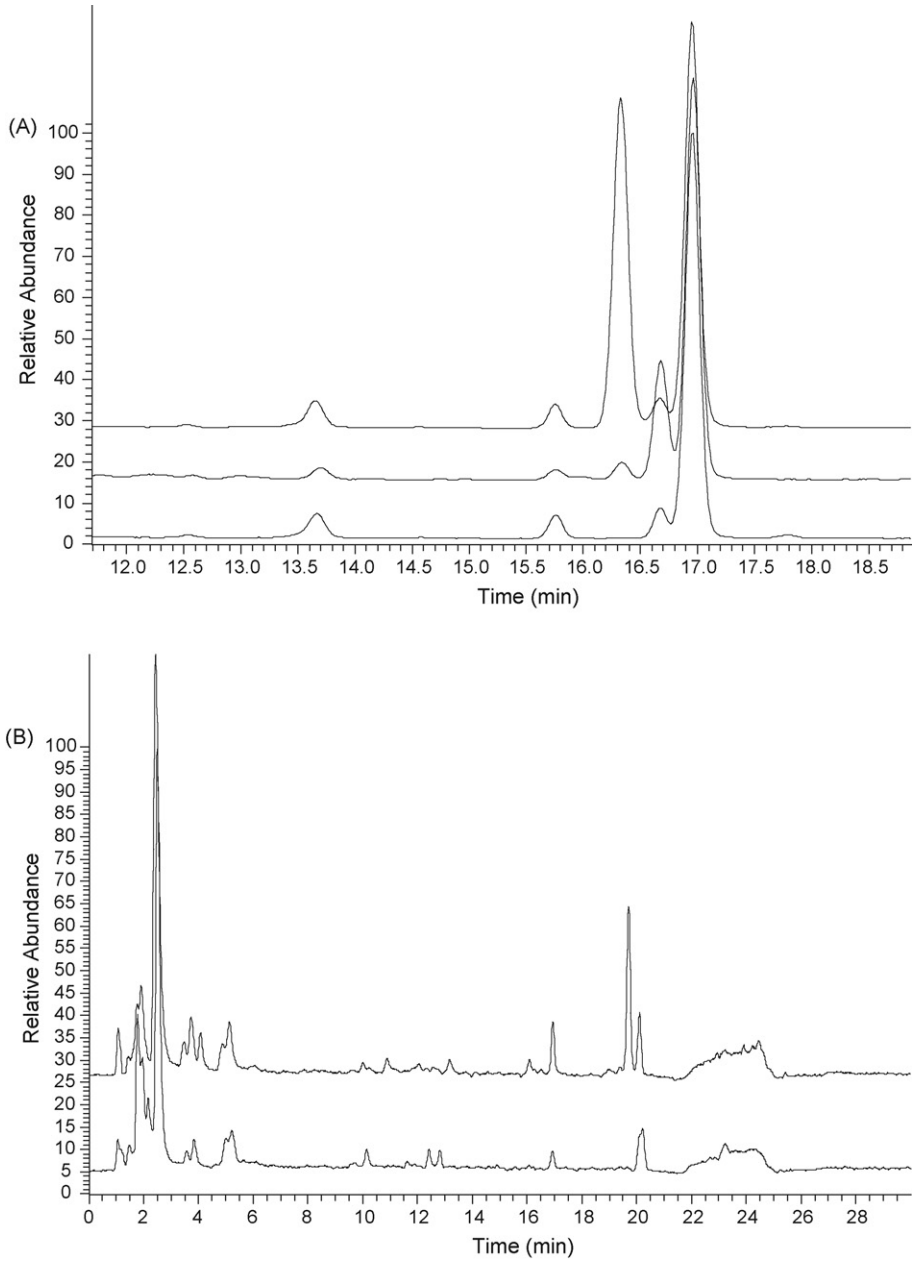

Fig. 2. Representative chromatograms monitoring (A) *m/z* 206 (DMB) and (B) *m/z* 220 (DMV internal standard). (A) The bottom chromatogram represents control rat plasma containing no DMB, the middle chromatogram represents control rat plasma containing 100 ng/ml DMB (16.3 min), and the top chromatogram represents control rat plasma containing 10,000 ng/ml DMB (16.3 min). (B) The bottom chromatogram represents control rat plasma containing no DMV internal standard and the top chromatogram represents control rat plasma containing 500 ng/ml DMV internal standard (19 min).

3.2.3. Accuracy and precision

FDA guidelines specify that the accuracies for all tested concentrations should be within ±15%, and the precisions, expressed by the coefficient of variation, should not exceed 15%, except for the LLQ, in which case these parameters should not exceed 20%. The accuracies and intra- and inter-assay precisions for the tested DMB concentrations (LLQ, QCL, QCM, and QCH) were all within those acceptance criteria (Table 2).

Table 2
Assay performance data for the quantitation of LLQ, QCL, QCM and QCH DMB concentrations in rat plasma

[DMB] (ng/ml)	Accuracy (%)	Intra-assay precision (%)	Inter-assay precision (%)	Replicates
100	97.3	5.11	5.52	15
200	94.6	5.14	4.8	15
1000	97.6	3.37	5.57	15
7500	107.4	5.17	6.17	15

Table 3
Stability of DMB

Storage condition	Concentration (ng/ml)		Stability (%)	CV (%)	Replicates
Stock solution 6 months					
4 °C	1,000,000		106.8	15.9	3
Plasma 3 freeze/thaw cycles					
−80 °C	QCL	200	99.5	5.9	3
	QCM	1,000	106.9	11.6	3
	QCH	7,500	97.6	4.4	3
Plasma 5 months					
−80 °C	QCL	200	101.4	7.6	3
	QCM	1,000	103.1	5.8	3
	QCH	7,500	112.5	10.1	3
Plasma 6 h					
Ambient temperature	QCL	200	97.4	0.6	3
	QCM	1,000	106.8	6.7	3
	QCH	7,500	99.8	1.5	3

3.2.4. Extraction recovery

Because the method developed includes a chemical reaction in plasma, ion suppression could not easily be assessed. However, the recovery of the assay was determined by adding DMB and DMV to the residue of 3 blank plasmas that had undergone acetonitrile precipitation and evaporation with the results from DMB and DMV added to 3 fresh plasmas before deproteination and evaporation. Recoveries of QCL and QCH concentrations from plasma were 77.5% (CV 10.4%) and 62.1% (CV 6.1%), respectively.

3.2.5. Stability

The stability of a DMB stock solution kept at 4 °C in the dark for 6 months was 106.8% (CV 15.9%) (Table 3). The stabilities of DMB stock, as well as QCL, QCM, and QCH solutions in plasma at room temperature for 6 h are shown in Table 3. The stabilities of DMB in plasma during freeze–thaw cycling and for 5 months at −80 °C are also shown in Table 3. The stability of extracted samples kept in the autosampler for more than 37 h, ranged from 86.1 to 108.3% and did not change in any consistent manner with time.

3.3. Application of the assay to biological samples

The assay proved well suited to the quantitation of DMB in plasma of rats treated orally with a 500 mg/kg dose of DMB sodium salt (equivalent to 414 mg/kg of free DMB). As shown in Fig. 3, maximum plasma DMB concentrations occurred at 2 h, but DMB concentrations were relatively constant at 400–600 μg/ml between 90 and 420 min after DMB administration. DMB concentrations in plasma decreased with a half-life of approximately 5 h (312 min), and DMB apparent clearance and volume of distribution was 1.45 ml/(min kg) and 0.65 l/kg, respectively. These pharmacokinetic data confirm the longer half-life of DMB compared to butyrate. In addition, the volume of distribution of DMB is similar to the volume of distribution reported for butyrate (0.48–0.72 l/kg) [25], lending support to the validity of our data and assay, and providing additional impetus for clinical evaluation of DMB.

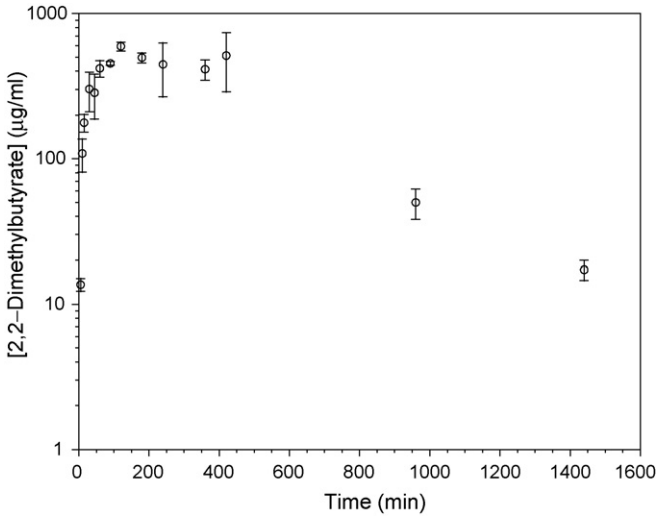

Fig. 3. Concentrations of DMB in plasma of rats treated orally with 500 mg/kg of sodium DMB. Symbols represent the means ± S.D. of samples from 3 rats at each time point.

4. Conclusion

Our objective was to develop and validate an analytical method for the quantitation of DMB in plasma. This was accomplished by employing a procedure that derivatizes carboxylic acids to amides followed by LC with mass spectrometric detection. The method developed overcomes the challenges associated with the polar nature of DMB, its low molecular weight, and its lack of a chromophore or fluorophore. The method proved suitable for assessing the pharmacokinetics of DMB in rats and was an important adjunct to the toxicology studies upon which clinical trials of DMB are being based. The validation procedures described for rat plasma are being applied to human plasma so that the method can be used in the clinical development of DMB for the treatment of β-thalassemia and sickle cell disease.

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References

[1] R. Redding-Lallinger, C. Knoll, Curr. Probl. Pediatr. Adolesc. Health Care 36 (2006) 346.
[2] D. Rund, E. Rachmilewitz, N. Engl. J. Med. 353 (2005) 1135.
[3] M.H. Steinberg, G.P. Rodgers, Medicine (Baltimore) 80 (2001) 328.

[4] M.J. Cunningham, E.A. Macklin, E.J. Neufeld, A.R. Cohen, Blood 104 (2004) 34.
[5] B. Wonke, Semin. Hematol. 38 (2001) 350.
[6] S.P. Perrine, Am. Soc. Hematol. Educ. Program. (2005) 38.
[7] E. Gallo, P. Massaro, R. Miniero, D. David, C. Tarella, Br. J. Haematol. 41 (1979) 211.
[8] S.P. Perrine, A. Rudolph, D.V. Faller, C. Roman, R.A. Cohen, S.J. Chen, Y.W. Kan, Proc. Natl. Acad. Sci. U.S.A. 85 (1988) 8540.
[9] E. Liakopoulou, C.A. Blau, Q. Li, B. Josephson, J.A. Wolf, B. Fournarakis, V. Raisys, G. Dover, T. Papayannopoulou, G. Stamatoyannopoulos, Blood 86 (1995) 3227.
[10] E. Liakopoulou, Q. Li, G. Stamatoyannopoulos, Blood Cells Mol. Dis. 29 (2002) 48.
[11] E. Skarpidi, H. Cao, B. Heltweg, B.F. White, R.L. Marhenke, M. Jung, G. Stamatoyannopoulos, Exp. Hematol. 31 (2003) 197.
[12] P. Constantoulakis, G. Knitter, G. Stamatoyannopoulos, Blood 74 (1989) 1963.
[13] S.P. Perrine, B.A. Miller, D.V. Faller, R.A. Cohen, E.P. Vichinsky, D. Hurst, B.H. Lubin, T. Papayannopoulou, Blood 74 (1989) 454.
[14] M.S. Boosalis, R. Bandyopadhyay, E.H. Bresnick, B.S. Pace, K. Van DeMark, B. Zhang, D.V. Faller, S.P. Perrine, Blood 97 (2001) 3259.
[15] B.S. Pace, G.L. White, G.J. Dover, M.S. Boosalis, D.V. Faller, S.P. Perrine, Blood 100 (2002) 4640.
[16] S. Castaneda, M.S. Boosalis, D. Emery, A. Thies, D.V. Faller, S.P. Perrine, Blood Cells Mol. Dis. 35 (2005) 217.
[17] A.F. Collins, H.A. Pearson, P. Giardina, K.T. McDonagh, S.W. Brusilow, G.J. Dover, Blood 85 (1995) 43.
[18] T. Ikuta, Y.W. Kan, P.S. Swerdlow, D.V. Faller, S.P. Perrine, Blood 92 (1998) 2924.
[19] G.J. Dover, S. Brusilow, S. Charache, Blood 84 (1994) 339.
[20] G.F. Atweh, M. Sutton, I. Nassif, V. Boosalis, G.J. Dover, S. Wallenstein, E. Wright, L. McMahon, G. Stamatoyannopoulos, D.V. Faller, S.P. Perrine, Blood 93 (1999) 1790.
[21] S.P. Perrine, S.A. Castaneda, M.S. Boosalis, G.L. White, B.M. Jones, R. Bohacek, Ann N.Y. Acad. Sci. 1054 (2005) 257.
[22] K.C. Yeh, K.C. Kwan, J. Pharmacokinet. Biopharm. 6 (1978) 79.
[23] M.L. Rocci Jr., W.J. Jusko, Comput. Programs Biomed. 16 (1983) 203.
[24] C.O. Kangani, D.E. Kelley, Tetrahedron Lett. 46 (2005) 8917.
[25] M.J. Egorin, Z.M. Yuan, D.L. Sentz, K. Plaisance, J.L. Eiseman, Cancer Chemother. Pharmacol. 43 (1999) 445.