ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
15 (1997) 973–981

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

# Mangafodipir trisodium injection, a new contrast medium for magnetic resonance imaging: detection and quantitation of the parent compound MnDPDP and metabolites in human plasma by high performance liquid chromatography

Kim Gunnar Toft *, Grete Arneberg Friisk, Tore Skotland

*Nycomed Imaging AS, Research and Development, P.O. Box 4220 Torshov, N-0401 Oslo, Norway*

Received 22 May 1996; accepted 27 August 1996

**Abstract**

Manganese(II) dipyridoxyl diphosphate (MnDPDP) is the active component of mangafodipir trisodium injection (Teslascan®), a new contrast medium for magnetic resonance imaging. A high performance liquid chromatography (HPLC) method was developed for the simultaneous determination of MnDPDP and its five major metabolites in human plasma, i.e. the dephosphorylation products MnDPMP (manganese(II) dipyridoxyl monophosphate) and MnPLED (manganese(II) dipyridoxyl ethylenediamine diacetate) and the corresponding substances obtained after transmetallation with zinc (ZnDPDP, ZnDPMP and ZnPLED). Heparinized blood samples from patients receiving mangafodipir trisodium injection were immediately mixed with solid trisodium phosphate dodecahydrate to obtain pH $10.0 \pm 0.2$ in order to inhibit further in vitro dephosphorylation and transmetallation. The plasma thus obtained was ultrafiltrated prior to HPLC analysis. The chromatographic separation was obtained using a mixed-bed resin with both anion exchange and reversed-phase functions (OmniPac™ PAX-500) using isocratic elution and UV detection at 310 nm. With an injection volume of 50 μl, the limit of quantitation (LOQ) values were 0.8–2.3 μM for the Mn compounds and 0.1–0.8 μM for the Zn compounds. The between-run accuracy of spiked plasma samples was in the range 97.5–106.7% with a precision in the range 3.1–9.0%. The best fit calibration curves were obtained using non-linear regression according to the equation $Y = A + BX^{M}$ in the concentration range from LOQ to 100 μM. Long-term storage of spiked plasma samples for three months at −20°C demonstrated the required stability with recovery values within 85–115% of MnDPDP and its five metabolites. © 1997 Elsevier Science B.V.

*Keywords:* HPLC; Mixed-bed resin; Mangafodipir trisodium; MnDPDP; Plasma

*Abbreviations:* DPDP, *N,N′*-dipyridoxylethylenediamine-*N,N′*-diacetate-5,5′-bis(phosphate), dipyridoxyl diphosphate; DPMP, *N,N′*-dipyridoxylethylenediamine-*N,N′*-diacetate-5-phosphate, dipyridoxyl monophosphate; PLED, *N,N′*-dipyridoxylethylenediamine-*N,N′*-diacetate, dipyridoxyl ethylenediamine diacetate; TSP, trisodium phosphate dodecahydrate.

* Corresponding author. Tel: +47 22891010; fax: +47 22891200.

0731-7085/97/$17.00 © 1997 Elsevier Science B.V. All rights reserved.
*PII* S0731-7085(96)01945-0

## 1. Introduction

Magnetic resonance imaging (MRI) is based on the varying signal intensity of different tissues. Because paramagnetic metal ions of gadolinium

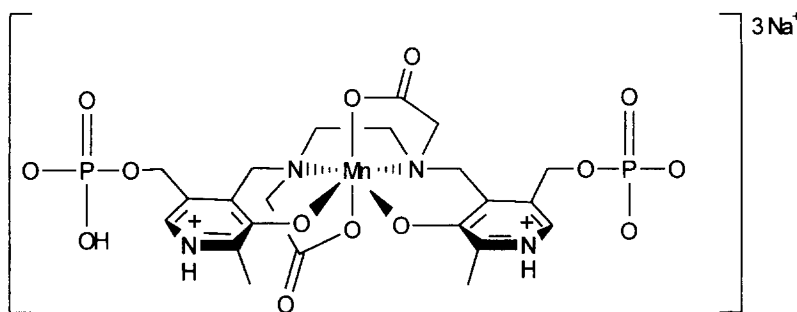


Fig. 1. Structural formula of the sodium salt of MnDPDP.

($Gd^{3+}$) and manganese ($Mn^{2+}$) alter the signal intensity of their surroundings by altering the relaxation times of hydrogen nuclei, they may be used as contrast media to improve the image quality and diagnostic utility of MRI [1]. These metals cannot, however, be administered as simple salts due to their toxicity and formation of precipitates with anions in the body. To be used as contrast media, they must be administered as chelated complexes [1]. Greatest focus has been on various chelates of Gd as contrast media for MRI [2,3]. This study is related to a new contrast medium based on Mn, and which has proven efficacy in both experimental animals [4,5] and man [6].

Mangafodipir trisodium injection (Teslascan®; a proprietary pharmaceutical formulation of Nycomed Imaging AS, Oslo, Norway) contains manganese(II)-*N*,*N*′-dipyridoxylethylenediamine-*N*,*N*′-diacetate-5,5′-bis(phosphate) (MnDPDP; structure shown in Fig. 1) as the active component. Metabolism of MnDPDP results in uptake of Mn in hepatocytes of normal liver parenchyma, but reduced or no uptake of Mn in metastases or focal liver lesions of hepatocellular origin, thus increasing the contrast and facilitating the detection of liver lesions [7]. A similar contrast effect may be obtained by injecting $MnCl_2$ [8], however, the effect of injecting Mn as MnDPDP is to obtain a tenfold decrease in toxicity of Mn [9].

In vitro experiments in human whole blood have shown that MnDPDP is metabolized by (i) dephosphorylation to the monophosphate manganese(II)-*N*,*N*′-dipyridoxylethylenediamine-*N*,*N*′-diacetate-5-phosphate (MnDPMP) and the fully dephosphorylated compound manganese (II)-*N*,*N*′-dipyridoxylethylenediamine-*N*,*N*′-diacetate (MnPLED) and (ii) simultaneous transmetallation to the corresponding zinc metabolites ZnDPDP, ZnDPMP and ZnPLED [10]. Mn found in the body exists in two different oxidation states, Mn(II) and Mn(III). In mangafodipir trisodium injection, Mn is maintained as Mn(II) by the presence of ascorbate and the absence of oxygen. Oxidation of Mn in MnDPDP or Mn metabolites may be a result of air exposure during sample handling, or due to endogenous compounds in plasma such as ceruloplasmin [11] promoting the conversion of Mn(II) to Mn(III).

The present paper describes the validation of a high performance liquid chromatography (HPLC) method for the simultaneous determination of MnDPDP and its five major metabolites in human plasma. A mixed-bed column resin with both anion exchange and reversed-phase functions was chosen for separation since it offered a satisfactory resolution between matrix components and the metabolites. Although the chromatographic profile showed relatively broad peaks, the separation proved sufficiently effective to give results suitable for pharmacokinetic calculations.

## 2. Materials and methods

### 2.1. Chemicals and reagents

MnDPDP, MnDPMP, MnPLED, ZnDPDP, ZnDPMP and ZnPLED were from Nycomed Imaging, Oslo, Norway. The synthesis and characterization of MnDPDP has been described by Rocklage et al. [12]. Human blood was collected from healthy caucasian male and female volunteers. Water was purified by reversed osmosis, ion exchange and filtration through an 0.45 μm filter using a Milli-Q system (Millipore, Bedford, MA). To minimize air oxidation of the Mn compounds water used to prepare calibration standards and control samples was degassed with $N_2$-gas for 30 min. Other chemicals were of analytical grade.

### 2.2. Instrumentation

The HPLC system consisted of a Spectra Physics SP 8800 ternary mobile phase pump, a Spectra Physics AS 3000 autosampler equipped with a Rheodyne 100 μl sample loop, and a Spectra Physics SP 8490 UV/VIS detector. The column was an OmniPac™ PAX-500, 4 × 250 mm, 8.5 μm (Dionex, Sunnyvale, CA) containing a mixed-bed resin of both anion exchange and reversed-phase functions with a guard column of 4 × 50 mm containing the same material.

### 2.3. Chromatographic conditions

Isocratic elution was used with 0.2 M sodium phosphate buffer containing 3% (v/v) acetonitrile as organic modifier and 1.5 mM tetrabutylammonium phosphate as ion pairing reagent. The pH in the mobile phase was adjusted with 1 M NaOH to pH 9.12. In order to obtain optimal separation of all six compounds it was necessary to adjust the acetonitrile concentration in the range 2.7–3.3% (v/v), when using different batches of the columns, or towards the end of the lifetime of the columns. The retention times of MnPLED and ZnPLED were very sensitive to acetonitrile concentration in the mobile phase, so the aqueous phase was degassed prior to adding the organic modifier. After connection to the HPLC system, the mobile phase was flushed vigorously with helium for a maximum 1 min and then a low constant supply of helium was applied during the chromatography to ensure that no oxygen was present. The mobile phase was stored at room temperature (20–23°C) and discarded after one week. The flow rate of the mobile phase was 1.2 ml min $^{-1}$ and the run time was 60 min. The separation was performed at room temperature, while the temperature in the autosampler was 4 ± 1°C. Injection volume was 50 μl and detection was at 310 nm, which is close to $\lambda_{max}$ for these substances.

### 2.4. Preparation of blood samples

Approximately 100 ml blood was collected in trace metal analysis tubes containing sodium heparin (Becton Dickinson, Meylan Cedex, France). The blood was pooled and adjusted to pH 10.0 by the addition of solid trisodium phosphate dodecahydrate (TSP) using a magnetic stirrer at low speed. The addition of TSP leads to a limited dehydration of the blood cells and thereby decreases the hematocrit value. This has to be corrected for when the 'true' plasma concentration of the metabolites are calculated. Some hemolysis may be observed but this does not affect the metabolic profile. Red cells were removed by centrifugation at 1500 × $g$ for 20 min at 4°C. Plasma was collected and transferred to Centriprep ultrafiltration devices (15 ml) with a cut-off of 30 kDa (Amicon, Beverly, MA). Ultrafiltration was performed in a fixed-angle rotor at 1500 × $g$ for 90 min at 4°C. The collected filtrate (called processed plasma) was pooled in a polyethylene bottle, purged with $N_2$-gas and stored at −20°C. Calibration standards and control samples were prepared from a stock solution containing 1 mM of each of the components MnDPDP, MnDPMP, MnPLED, ZnDPDP, ZnDPMP and ZnPLED in processed plasma. All samples were purged with $N_2$-gas and stored at −20°C. They were thawed at 4°C, placed in the autosampler and analyzed within 6 h.

Blood samples from patients receiving mangafodipir trisodium injection were collected in trace metal analysis tubes containing sodium hep-

arin, and 1 ml of blood was transferred to a polyethylene tube containing enough TSP to adjust the pH of the blood to pH $10.0 \pm 0.2$ (approximately 17 mg $ml^{-1}$). Because of individual pH variation of the blood, the amount of TSP had to be determined by collecting blood from each person prior to giving the contrast medium. Blood and TSP were mixed either by gentle inversion of the tubes or by using a vial roller. Plasma was collected as described above and ultrafiltrated by using a Centricon ultrafiltration device (2 ml) with a cut-off of 30 kDa (Amicon, Beverly, MA). Ultrafiltration was performed in a fixed-angle rotor at $5000 \times g$ for 60 min at 4°C. The collected filtrate (processed plasma) was stored at −20°C for up to three months.

The recovery of MnDPDP and metabolites after ultrafiltration was determined by preparing control samples in plasma adjusted to pH 10 with TSP and spiked with MnDPDP and the five metabolites at concentrations of 5, 50 and 100 μM. Ultrafiltration was performed as described above and the recovery was calculated from calibration curves in processed plasma.

### 2.5. Determination of the limit of quantitation

To determine the limit of quantitation (LOQ), a dilution array in processed plasma containing MnDPDP and the five main metabolites was analysed with six replicate analyses at each of the following concentrations; 10, 5, 4, 3, 2.5, 1, 0.5, 0.25, 0.125 μM and blank. The LOQs for the Zn compounds were defined as $LOQ = X + 10*SD_X$, where $X$ was the mean area response of the endogenous peak in the blank sample with the same retention time as the actual metabolite and $SD_X$ was the S.D. of the mean area response of the endogenous peak [13]. For the Mn metabolites where no endogenous peak with the same retention time as the actual metabolite could be detected, the LOQs were defined as $LOQ = 10*SD_{0.125}$, where $SD_{0.125}$ was the S.D. of the mean area response of the 0.125 μM sample. At this concentration, the signal:noise ratio for the Mn metabolites was approximately 3.

### 2.6. Data handling

PE Nelson ACCESS*CHROME GC/LC data sampling system, v. 1.8, was used for sampling and integration of the chromatograms, and for calculating the capacity factors ($k'$) and resolution factors ($R_S$). GraphPad Inplot, v. 4.03, was used for the preparation of calibration curves and calculating the metabolite concentrations. Lotus 1-2-3, v. 3.1, or Excel, v. 5.0, were used for calculating the mean, S.D., S.E.M., R.S.D. and R.S.E.M.

## 3. Results and discussion

Sample preparation is a very important step in the handling of blood samples obtained after injection of mangafodipir trisodium injection, as it is essential to stop further dephosphorylation and transmetallation of the metabolites in vitro [10]. The addition of TSP prevents further dephosphorylation by inhibiting the phosphatases, and prevents further transmetallation by raising the pH. Ultrafiltration removes degradative enzymes and oxidizing components like ceruloplasmin, and thereby contributes to stop further biotransformation. The recovery after ultrafiltration, using a Centriprep ultrafiltration device with a MW cut-off of 30 kDa, of 5, 50 and 100 μM MnDPDP spiked into plasma was $85.9 \pm 0.6$, $102.8 \pm 10.8$ and $113.3 \pm 2.4\%$ (mean ± S.D., $n = 3$) respectively. Similar recovery values were obtained with the five metabolites (data not shown). Several other ultrafiltration devices were also tested; Centricon with a cut-off of 30 kDa, Ultrafree$^R$-MC with a cut-off of 30 kDa (Millipore, Bedford, MA) and Centrisart with a cut-off of 20 kDa (Sartorius, Göttingen, Germany), and all of them showed recovery values within ±15% of the added amount (data not shown).

The selectivity of the HPLC method was demonstrated by verifying that no major matrix component of blank processed plasma prepared from several blood donors eluted at the expected retention times ($t_R$) for MnDPDP and the five metabolites (Fig. 2). The chromatographic peak separation of MnDPDP and its metabolites was determined by calculation of the capacity factor

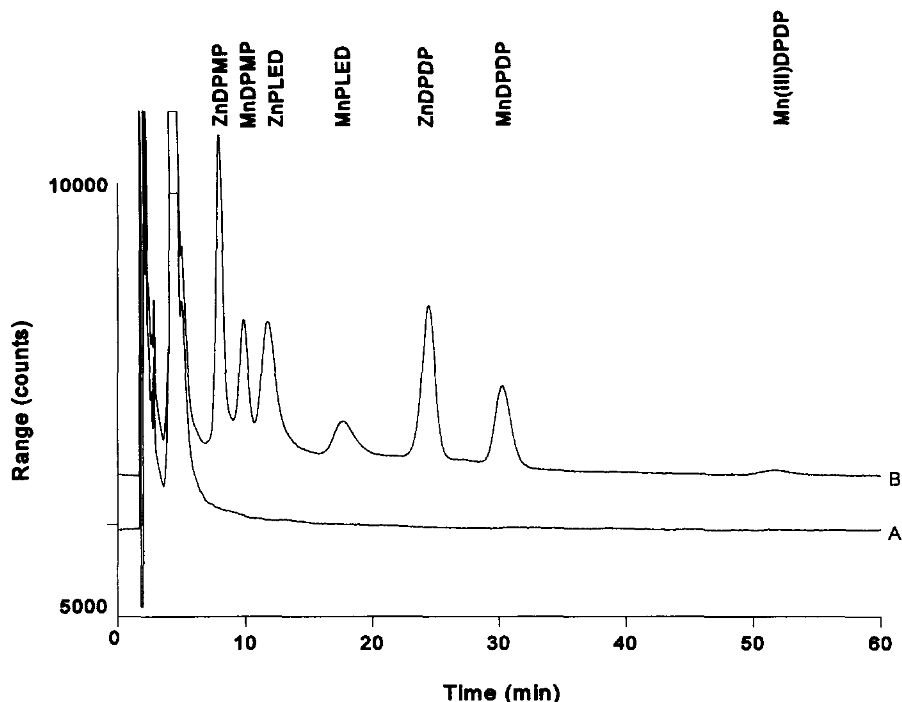


Fig. 2. Chromatogram of: (A), blank processed plasma; and (B), processed plasma spiked with approximately 4 μM of MnDPDP, MnDPMP, MnPLED, ZnDPDP, ZnDPMP and ZnPLED. Analysis was performed by isocratic elution in 0.2 M sodium phosphate pH 9.12 containing 3% (v/v) acetonitrile and 1.5 mM tetrabutylammonium phosphate at a flow rate of 1.2 ml $min^{-1}$ and UV detection at 310 nm.

($k'$) and resolution ($R_S$) in control samples containing approximately 4 μM and 12 μM of each of the six components. The results from the calculation of the capacity factors (Table 1) showed that the concentration range of MnDPDP and the five metabolites were well within the linear capacity of the column, which is the critical sample size above which sample retention time data can no longer be used for qualitative analysis (comparison with $t_R$ values of known components) [14]. The resolution between adjacent peaks were above 1.6 except between MnDPMP and ZnPLED where the $R_S$-value was 0.96 (Table 2).

Table 1
The capacity factors ($k'$) for MnDPDP, MnDPMP, MnPLED, ZnDPDP, ZnDPMP and ZnPLED

| Compound | $k'$ | R.S.D. (%) |
|---|---|---|
| ZnDPMP | 3.00 | 2.7 |
| MnDPMP | 3.95 | 2.5 |
| ZnPLED | 4.80 | 3.1 |
| MnPLED | 7.62 | 3.5 |
| ZnDPDP | 11.37 | 3.4 |
| MnDPDP | 14.29 | 3.4 |

Estimated from control samples containing 4 and 12 μM of the compounds in processed plasma. The figures are given as the mean of 52 independent analyses within 7 analytical series.

Table 2
The resolution factors ($R_S$) between adjacent peaks

| Compounds | $R_S$ | R.S.D. (%) |
|---|---|---|
| ZnDPMP/MnDPMP | 1.61 | 2.5 |
| MnDPMP/ZnPLED | 0.96 | 9.4 |
| ZnPLED/MnPLED | 2.07 | 5.8 |
| MnPLED/ZnDPDP | 2.83 | 10.6 |
| ZnDPDP/MnDPDP | 2.67 | 1.9 |

Estimated from control samples containing 4 and 12 μM of the compounds in processed plasma. The figures are given as the mean of 52 independent analyses within 7 analytical series.

Minor amounts (up to a total of approximately 10%) of the chelates of oxidized manganese, Mn(III)PLED, Mn(III)DPMP and Mn(III)DPDP, were observed in some of the analytical runs. These oxidized compounds were identified by analyzing separate standards, and they eluted in processed plasma with capacity factors of 9.9, 12.5 and 25.4, respectively. Oxidation was especially pronounced if no precautions were taken to avoid it. For example; the water used for the preparation of control samples and calibration standards should be properly degassed and purged with $N_2$. Moreover, addition of too much TSP to the blood samples should be avoided as higher pH favors oxidation; plasma samples should be ultrafiltrated as soon as possible and the ultrafiltrate immediately frozen at −20°C. The thawed samples should be analyzed within 6 h when kept at 4°C. Both oxidation, transmetallation with Zn, and dephosphorylation were more pronounced when the samples were stored at 23°C than at 4°C. The results described above indicate that the minor amount of Mn(III) compounds detected did not represent true metabolites of Mn(II)DPDP, but rather represented artefacts arising from oxidation during sample handling.

With an injection volume of 50 μl, the LOQ were 0.95, 0.83 and 2.28 μM for MnDPDP, MnDPMP and MnPLED, respectively, and 0.78, 0.10 and 0.31 μM for ZnDPDP, ZnDPMP and ZnPLED, respectively. The difference in LOQ values are only to a minor extent due to different light absorption of the compounds, as the molar absorption coefficient for the Mn and Zn compounds were found to be approximately 11 000 and 13 000 $M^{-1}$ $cm^{-1}$, respectively.

For practical reasons, the determination of the LOQs was carried out with equimolar quantities of MnDPDP and its metabolites in the samples. After i.v. administration of mangafodipir trisodium injection, however, the individual concentration of the metabolites may vary considerably. Consequently, when calculating pharmacokinetic values, one has to reconsider the LOQ values on the basis of the specific metabolites present in the plasma samples. This is especially true for the peaks with the lowest resolution factor MnDPMP and ZnPLED, which have an $R_S$ of approximately 1.0 (Table 2). Theoretically, when the peak area ratio of two adjacent peaks with an $R_S = 1.0$ varies from 3:1 to 1:3, the accuracy in quantitating by peak area is still ±3% [14]. Thus, the difference in LOQ for MnDPMP and ZnPLED will be affected only to a minor extent within a variation in the relative concentration of the two compounds by a factor of 9.

Table 3
The estimated regression parameters (mean ± S.E.) of the equation $Y = A + BX^M$

| Compound | $A$ | $B$ | $M$ |
|---|---|---|---|
| MnDPDP | 4321 ± 9049 | 17404 ± 5147 | 1.07 ± 0.04 |
| MnDPMP | −5200 ± 3312 | 14351 ± 4862 | 1.06 ± 0.10 |
| MnPLED | −2168 ± 2981 | 7631 ± 4330 | 1.26 ± 0.15 |
| ZnDPDP | 4453 ± 8106 | 33234 ± 4666 | 1.05 ± 0.05 |
| ZnDPMP | −494 ± 4218 | 29556 ± 1664 | 1.01 ± 0.01 |
| ZnPLED | −2371 ± 5518 | 29014 ± 5957 | 1.08 ± 0.04 |

Based on the analysis of 6 different series of processed plasma spiked with MnDPDP, MnDPMP, MnPLED, ZnDPDP, ZnDPMP and ZnPLED in concentrations 0.25, 0.50, 1.0, 2.5, 5, 10, 20 and 40 μM. Each concentration was analysed in duplicate.

In a phase I trial with male volunteers administered mangafodipir trisodium injection at doses of 5 or 10 μmol MnDPDP $kg^{-1}$ body weight, the maximum concentrations of individual metabolites in plasma were found to be below 40 μM (to be published). The best fit calibration curves in the range LOQ to 40 μM were obtained by using non-linear regression analyses according to the equation $Y = A + BX^M$. No weighting factor was used. Table 3 shows the estimated regression parameters for the calibration curves. The degree of non-linearity, $M$, varied between 1.01 and 1.08, except for MnPLED which showed an $M$-value of 1.26. For a linear calibration curve, the factor $M$ equals 1, and a deviation from this value implies a certain degree of curvature or non-linearity. The reverse calculated mean of the six components were within the range 85–115% in the whole concentration range of the calibration curves. Similar figures as those presented in Table 3 were obtained for regression parameters for calibration curves in the range LOQ–100 μM (data not shown). The results showed that the calibration curves were sufficiently reproducible.

Table 4
The between-run accuracy and precision of the method

| Compound | Spiked concentration (μM) | Found concentration (μM) | Recovery (%) | R.S.E.M. (%) |
|---|---|---|---|---|
| MnDPDP | 5.2 | 5.2 | 100.5 | 4.4 |
| | 51.8 | 50.5 | 97.6 | 6.8 |
| | 103.5 | 105 | 101.4 | 3.2 |
| MnDPMP | 4.6 | 4.5 | 98.4 | 6.9 |
| | 45.8 | 45.5 | 99.5 | 6.5 |
| | 91.5 | 95.1 | 103.9 | 5.8 |
| MnPLED | 5.2 | 5.1 | 97.5 | 7.4 |
| | 52.3 | 54.1 | 103.4 | 5.3 |
| | 104.6 | 108 | 103.0 | 4.5 |
| ZnDPDP | 5.0 | 5.2 | 104.8 | 9.0 |
| | 49.6 | 51.6 | 104.0 | 5.9 |
| | 99.2 | 106 | 106.6 | 3.1 |
| ZnDPMP | 5.0 | 4.9 | 97.5 | 7.1 |
| | 50.3 | 52.7 | 104.9 | 4.6 |
| | 100.5 | 106 | 105.1 | 4.0 |
| ZnPLED | 5.1 | 5.4 | 105.9 | 7.0 |
| | 51.0 | 53.2 | 104.3 | 4.7 |
| | 102.0 | 109 | 106.7 | 3.6 |

Measured in plasma samples spiked with MnDPDP, MnDPMP, MnPLED, ZnDPDP, ZnDPMP and ZnPLED in three different concentrations. Analyses were performed on three successive days with three independent parallels and two replicate analyses of each.

The within-run accuracy and precision were determined by measuring the % recovery and % R.S.D., respectively, in processed plasma samples spiked with 5, 50 and 100 μM of MnDPDP and the five metabolites. Three independent parallels and two replicate analyses of each were analysed within the same analytical run. The overall accuracy was in the range 89.9–113.2% and the overall precision was in the range 0.3–5.7%. The between-run accuracy and precision were determined by measuring the % recovery and % R.S.E.M., respectively, in processed plasma samples spiked with 5, 50 and 100 μM of MnDPDP and the five metabolites. Three independent parallels and two replicate analyses of each were analysed within the same analytical run on three successive days. The accuracy for all components was in the range 97.5–106.7% recovery and the precision was in the range 3.1–9.0% R.S.E.M. (Table 4). The data showed that the contribution of bias from day to day variation was insignificant. Although the lowest concentration used in the formal accuracy and precision study was approximately 5 times the LOQ value, other experiments showed an acceptable precision ( < 20% R.S.D.) at the actual LOQ values. Six replicate analyses of processed plasma samples spiked with MnDPDP and the five metabolites showed a % R.S.D. in the measured peak area at the respective LOQ values of 9.4% for MnDPDP, 8.5% for MnDPMP, 7.7% for MnPLED, 3.6% for ZnDPDP, 4.3% for ZnDPMP and 6.8% for ZnPLED.

Results from a long-term stability study showed that MnDPDP, MnDPMP, MnPLED, ZnDPDP, ZnDPMP and ZnPLED spiked into processed plasma at the concentrations 4, 12 and 30 μM were stable for 82 days when stored at −20°C (Table 5).

In conclusion, the HPLC method developed for separation and analysis of MnDPDP and the metabolites MnDPMP, MnPLED, ZnDPDP, ZnDPMP and ZnPLED in plasma provides a sensitive, accurate and reproducible analytical procedure. The method is suitable for analyses of

Table 5
Plasma samples spiked with MnDPDP, MnDPMP, MnPLED, ZnDPDP, ZnDPMP and ZnPLED in three different concentrations were stored at −20°C for the number of days indicated

| Compound | Spiked concentration (μM) | % Recovery of spiked sample (mean ± S.E.) | | | |
|---|---|---|---|---|---|
| | | Day 0 ($n = 6$) | Day 30 ($n = 4$) | Day 58 ($n = 4$) | Day 82 ($n = 4$) |
| MnDPDP | 4.38 | 94.6 ± 3.7 | 100.1 ± 1.3 | 93.6 ± 1.6 | 89.8 ± 3.0 |
| | 13.1 | 92.0 ± 5.0 | 95.7 ± 0.6 | 101.3 ± 0.8 | 94.1 ± 2.0 |
| | 32.8 | 95.6 ± 1.3 | 97.0 ± 0.4 | 98.7 ± 1.0 | 97.1 ± 0.7 |
| MnDPMP | 4.36 | 93.5 ± 1.2 | 111.7 ± 1.9 | 95.8 ± 1.8 | 99.7 ± 4.4 |
| | 13.1 | 97.2 ± 5.0 | 108.1 ± 0.5 | 101.7 ± 1.2 | 110.9 ± 2.4 |
| | 32.7 | 103.7 ± 1.1 | 107.5 ± 0.6 | 102.3 ± 0.7 | 124.4 ± 1.1 |
| MnPLED | 4.67 | 81.6 ± 2.1 | 117.0 ± 1.4 | 93.1 ± 2.5 | 93.0 ± 7.5 |
| | 14.0 | 85.6 ± 3.9 | 117.0 ± 2.6 | 96.5 ± 0.6 | 103.1 ± 1.4 |
| | 35.0 | 91.7 ± 1.2 | 111.8 ± 0.4 | 97.1 ± 0.6 | 98.8 ± 0.8 |
| ZnDPDP | 4.36 | 90.7 ± 2.5 | 98.0 ± 1.4 | 95.8 ± 0.7 | 101.0 ± 5.4 |
| | 13.1 | 92.6 ± 4.1 | 94.7 ± 0.7 | 92.2 ± 0.3 | 97.0 ± 1.6 |
| | 32.7 | 92.8 ± 1.1 | 88.8 ± 0.3 | 95.1 ± 0.8 | 96.0 ± 0.6 |
| ZnDPMP | 4.51 | 96.4 ± 0.7 | 98.8 ± 0.8 | 92.0 ± 0.7 | 96.4 ± 0.9 |
| | 13.5 | 98.9 ± 4.6 | 96.3 ± 0.3 | 94.2 ± 0.8 | 94.7 ± 0.4 |
| | 33.8 | 100.0 ± 1.1 | 96.3 ± 0.4 | 95.1 ± 0.6 | 95.6 ± 0.4 |
| ZnPLED | 4.07 | 89.3 ± 1.7 | 107.8 ± 0.9 | 96.1 ± 1.0 | 100.7 ± 3.3 |
| | 12.2 | 94.3 ± 4.3 | 105.5 ± 0.9 | 95.7 ± 1.4 | 97.0 ± 0.6 |
| | 30.6 | 97.6 ± 1.0 | 103.4 ± 0.5 | 97.9 ± 0.7 | 93.3 ± 1.0 |

The stability of the samples was measured as mean ± S.E. of spiked concentrations. The number of independent parallels are shown in parentheses. Each parallel was analysed in duplicate

MnDPDP and metabolites in plasma after intravenous administration of mangafodipir trisodium injection to patients.

## Acknowledgements

The contributions made in the early phase of this study by Jan Ji Lai and Gene Jamieson are greatly appreciated.

## References

[1] A.D. Watson and S.M. Rocklage, in C.B. Higgins, H. Hricak and C.A. Helms (Eds.), Magnetic Resonance Imaging of the Body, 2nd edn, Raven Press, New York, 1992, pp. 1257–1287.
[2] H.P. Niendorf, J.C. Dinger, J. Haustein, I. Cornelius, A. Alhassan and W. Clauss, Eur. J. Radiol., 13 (1991) 15–20.
[3] B. Lundby and P. Gordon, Eur. J. Clin. Res., 8 (1996) 37–48.
[4] Y. Ni, G. Marchal, X. Zhang et al., Invest. Radiol., 28 (1993) 520–528.
[5] R.C. Nelson, J.L. Chezmar, L.B. Newberry, J.A. Malko, R.K. Gedgaudas-McClees and M.E. Bernardino, Invest. Radiol., 26 (1991) 569–573.
[6] E. Rummeny, Ch. Ehrenheim, H.B. Gehl et al., Invest. Radiol., 26(Suppl. 1) (1991) S142–S145.
[7] N.M. Rofsky, J.C. Weinreb, M.E. Bernardino, S.W. Young, J.K.T. Lee and M.E. Noz, Radiology, 188 (1993) 53–59.
[8] G. Elizondo, C.J. Fretz, D.D. Stark et al., Radiology, 178 (1991) 73–78.
[9] P. Jynge, H. Brurok, A. Asplund et al., in P.A. Rinck and R.N. Muller (Eds), New Developments in Contrast Agent Research, European Magnetic Resonance Forum, Mons, Belgium, 1995, pp. 83–103.
[10] K.G. Toft, G.M. Kindberg and T. Skotland, J. Pharmaceut. Biomed. Anal., 15 (1997) 983–988.
[11] J.W. Critchfield and C.L. Keen, Metabolism, 41 (1992) 1087–1092.
[12] S.M. Rocklage, W.P. Cacheris, S.C. Quay, F.E. Hahn and K.N. Raymond, Inorg. Chem., 28 (1989) 477–485.

[13] J.K. Taylor, Quality Assurance of Chemical Measurements, 3rd Printing, Lewis Publishers, Chelsea, MI, 1988, pp. 78–82.

[14] L.R. Snyder and J.J. Kirkland, Introduction to Modern Liquid Chromatography, 2nd edn, Wiley, New York, 1979, pp. 16–80.