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Journal of Pharmaceutical and Biomedical Analysis
25 (2001) 109–114

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Determination of mangafodipir trisodium and related impurities in bulk substance and pharmaceutical formulation by ion-pair high-performance liquid chromatography

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Received 4 November 1999; received in revised form 14 September 2000; accepted 20 September 2000

Abstract

The development of an ion-pair liquid chromatographic method for determination of mangafodipir trisodium and related impurities is described. Good resolution was obtained when using a polymeric reverse-phase column and a mobile phase of pH* 10.5 composed by borate buffer, acetonitrile, and tetrabutylammonium hydrogensulphate as ion pair agent. Validation of the method showed good selectivity, precision, accuracy and linearity, and detection limits of 0.1–0.2 μg/ml. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Mangafodipir trisodium; MnDPDP; Ion-pair liquid chromatography; High-performance liquid chromatography

1. Introduction

The paramagnetic manganese chelate mangafodipir trisodium (manganese dipyridoxyl diphosphate sodium salt) (MnDPDP SS) is a new contrast enhancing agent for magnetic resonance imaging of the liver [1] formed by complexation of manganese ion (Mn^{2+}) with the organic ligand fodipir (dipyridoxyl diphosphate). The synthesis and characterisation of MnDPDP SS have been described previously [2,3]. The pharmaceutical formulation TESLASCAN™ 0.01 mmol/ml (Nycomed Amersham) contains ascorbic acid and sodium chloride in addition to MnDPDP SS.

The impurities detected in mangafodipir trisodium drug substance include the by-products denoted MnDPDP-MOA and Mn(5-methyl)-DPMP, the hydrolytic degradation product MnDPMP, and the oxidative degradation product Mn(III)DPDP. MnDPMP is also the major degradation product formed during heat treatment and storage of the pharmaceutical formulation. Structural formulae of sodium salts of MnDPDP and related impurities are presented in Fig. 1.

Chromatographic analysis of metal chelates may be performed with ion-exchange [4] or ion-pair high-performance liquid chromatography (HPLC) [5,6]. Preliminary tests revealed that ion-pair HPLC was more robust with respect to

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PII: S0731-7085(00)00500-8

column stability and lifetime than ion-exchange HPLC, and therefore this technique was chosen.

In the present paper, an ion-pair HPLC method for determination of MnDPDP and related impurities in mangafodipir trisodium drug substance and pharmaceutical formulation is described.

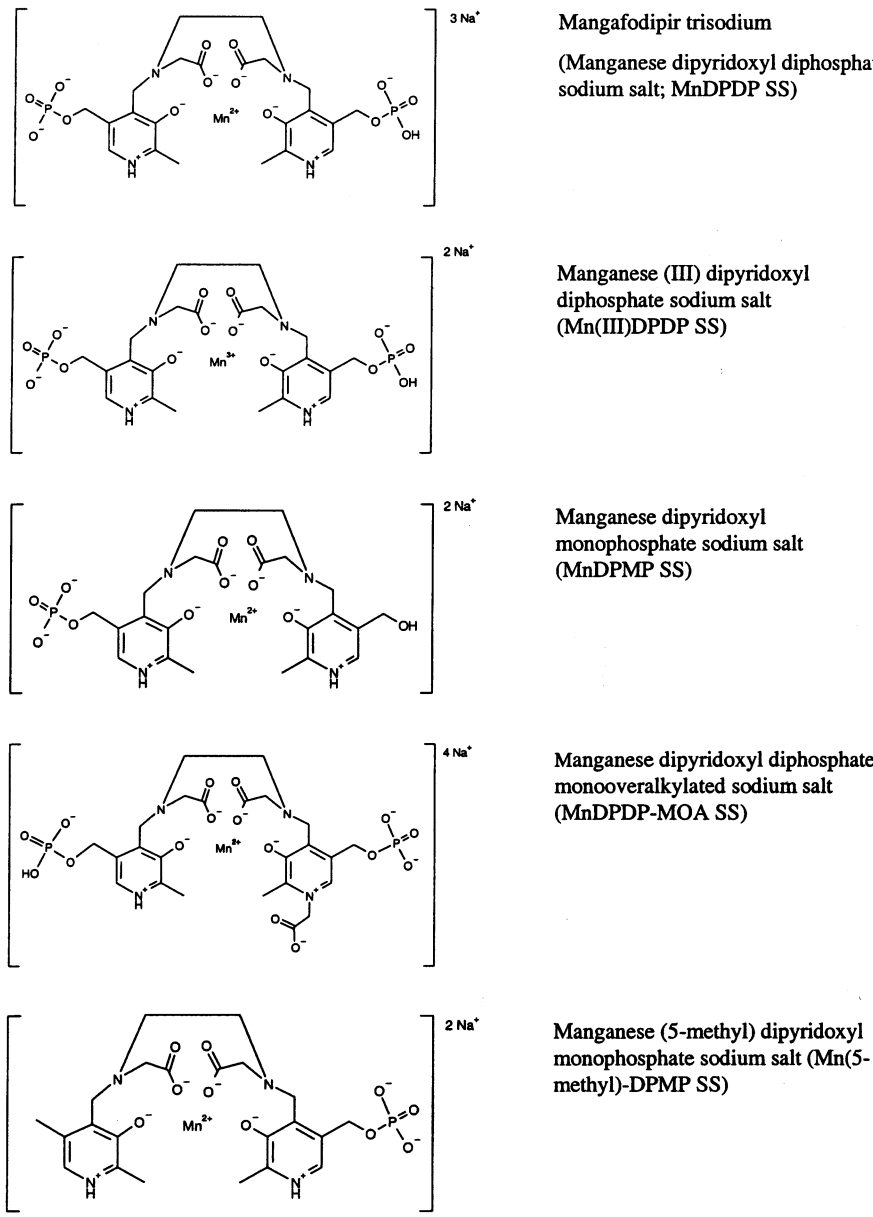

Fig. 1. Structural formulae of sodium salts of mangafodipir trisodium and related impurities.

2. Experimental

2.1. Materials

Disodium hydrogenphosphate heptahydrate, boric acid, sodium hydroxide, hydrochloric acid, ascorbic acid (all p.a. grade) and acetonitrile (for chromatography) were purchased from Merck (Darmstadt, Germany). Tetrabutylammonium hydrogensulphate (puriss.) and tetrahexylammonium hydrogensulphate (purum) were purchased from Fluka (Buchs, Switzerland). MnDPDP SS, MnDPMP SS, MnDPDP-MOA SS, Mn(III)DPDP SS and Mn(5-methyl)-DPMP SS (see Fig. 1 for names and structural formulae) were produced by Nycomed Imaging R&D (Oslo, Norway).

2.2. Chromatography

The HPLC system consisted of an ISS-100 autosampler and a Model 620 pump from Perkin Elmer (Norwalk, CT, USA), a SpectraFocus or a Spectra System UV 1000 UV/VIS-detector from Thermo Separation Products (Fremont, CA, USA), and the Access*Chrom integration system (Perkin Elmer Nelson, Cupertino, CA, USA). A 150 × 4.6 mm i.d. polymeric reverse phase PRP-1 5 U column with PEEK body was used (Hamilton Company, Reno, NV, USA). The injection volume was 10 μl, the mobile phase flow rate was 0.8 ml/min, the column temperature was 20°C, and the column effluent was monitored at 310 nm (UV).

The other HPLC columns tested were: Jordi RP 100 Å, 250 × 4.6 mm i.d. PEEK body; Hema RP C8, 250 × 4.6 mm i.d. PEEK body; and Interaction Poly RP-C0, 150 × 4.6 mm i.d. PEEK body (all columns obtained from Alltech, Deerfield, IL, USA); Asahipak ODP-50, 150 × 4.6 mm i.d. (Asahi, Kawasaki-shi, Japan); and Supelcosil LC-8, 250 × 4.6 mm i.d. (Supelco, Bellefonte, PA, USA).

2.3. Solutions

All solutions were made with water purified by reverse osmosis and ion exchange, which was bubbled with nitrogen for at least 30 min before use to remove dissolved oxygen.

2.3.1. Acetonitrile-borate/tetrabutylammonium hydrogensulphate (pH 9.9; 13 mM borate, 36 mM TBA) (25:75, v/v) mobile phase

Boric acid (0.61 g) and tetrabutylammonium hydrogensulphate (TBA) (9.2 g) were dissolved in 640 ml water in a 1 l volumetric flask. The pH was adjusted to about 9.9 with NaOH. After filtration through a 0.45 μm filter, 250 ml acetonitrile was added (giving pH* about 10.5), and water was added to 1.0 l. The mobile phase was mixed by repeated inversion and degassed prior to use.

2.3.2. Phosphate buffer (pH 8.0; 0.1 M)

Disodium hydrogenphosphate heptahydrate (26.8 g) was dissolved in 900 ml water in a 1 l volumetric flask. The pH was adjusted to 8.0 with 1 M sodium hydroxide or 1 M hydrochloric acid as required. The solution was diluted to volume with water, mixed, filtered through a 0.45 μm filter and degassed prior to use.

2.3.3. Standard stock solutions

To make an impurity standard stock solution, 10 mg each of MnDPMP SS, Mn(5-methyl)-DPMP SS and MnDPDP-MOA SS were weighed into a 100 ml volumetric flask and diluted to volume with water. To make a MnDPDP SS stock solution, 100 mg was weighed into a 50 ml volumetric flask and diluted to volume with water. A MnDPDP SS check standard stock solution was made by weighing 80 mg into a 50 ml volumetric flask and diluted to volume with water.

2.3.4. Standard mixture (MnDPDP SS, 0.40 mg/ml; MnDPMP SS, 0.01 mg/ml; Mn(5-methyl)-DPMP SS, 0.01 mg/ml; and MnDPDP-MOA SS, 0.01 mg/ml)

The standard mixture was prepared by transferring 5.0 ml impurity standard stock solution, 10.0 ml MnDPDP SS stock solution, and 5.0 ml phosphate buffer to a 50 ml volumetric flask and diluting to volume with water.

2.3.5. MnDPDP SS check standard (0.32 mg/ml)

To make the check standard, 10.0 ml check standard stock solution was transferred to a 50 ml volumetric flask, 5.0 ml phosphate buffer was added, and water added to volume.

2.4. Sample preparation

For drug substance, 100 ± 10 mg (anhydrous basis) was weighed into a 50 ml volumetric flask and diluted to volume with water. Ten milliliters were transferred to a new 50 ml volumetric flask, 5 ml phosphate buffer was added, and water added to volume. For the pharmaceutical formulation (7.6 mg/ml), 5.0 ml was transferred to a 100 ml volumetric flask, 10.0 ml phosphate buffer added, and water added to volume.

2.5. Analysis of standards and samples

Sample and standard solutions were transferred to 1.5 ml autosampler vials. MnDPDP SS was quantified by one-point external standard calibration. Injections of check standard and sample solutions were bracketed by injection of standards. Impurities were quantified using area normalisation, and results given as percent area.

3. Results and discussion

3.1. Method development

A silica-based reverse-phase column and methanol/water mobile phases with TBA as ion pairing agent and phosphate buffer pH 6.8 were tested. No acceptable chromatograms were obtained. Therefore mobile phases with higher pH were prepared. Borate was used as buffer, and acetonitrile was used instead of methanol. Five types of polymeric HPLC columns were tested, and three column types were found to be suitable: Hamilton PRP-1, Jordi RP 100 Å and Asahipak ODP-50. The Hamilton and Asahipak columns gave the best resolution; however, the Hamilton column was chosen because it was available in a nonmetal body (PEEK).

The ion-pair agents TBA and tetrahexylammonium hydrogensulphate (THA) were tested. Both agents gave good results; however, a higher concentration of acetonitrile had to be used when employing THA. Therefore TBA was chosen.

Method optimalisation was performed using a standard mixture containing MnDPDP SS, MnDPMP SS and MnDPDP-MOA SS, and samples of drug substance and pharmaceutical formulation that were stressed by storage at high temperature and humidity. These samples contained Mn(5-methyl)-DPMP SS and Mn(III)DPDP SS in addition to the compounds already mentioned, as well as some unidentified compounds.

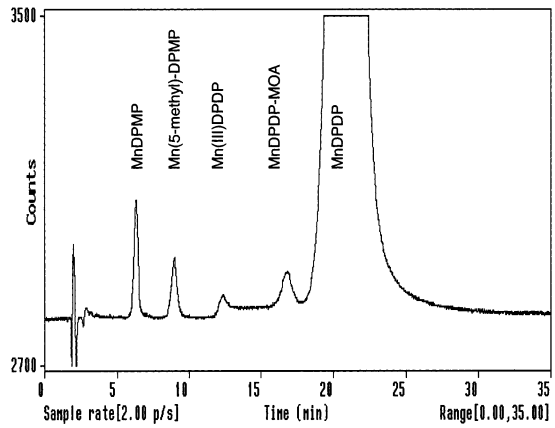

Fig. 2. Liquid chromatogram of a solution of MnDPDP SS (0.4 mg/ml) containing Mn(III)DPDP SS (0.4 μg/ml), and spiked with MnDPMP SS (1.9 μg/ml), Mn(5-methyl)-DPMP SS (1.6 μg/ml) and MnDPDP-MOA SS (2.0 μg/ml).

When increasing the TBA concentration of the mobile phase, the retention of MnDPDP-MOA relative to MnDPDP SS was found to decrease, while the relative retention of other compounds remained fairly unchanged.

When increasing the pH of the mobile phase, the relative retention of MnDPDP-MOA, Mn(5-methyl)-DPMP and Mn(III)DPDP were found to decrease.

The pH and concentrations of TBA were chosen in order to obtain optimal resolution for known and unknown impurities, and the concentration of acetonitrile was chosen to give a retention of the most strongly retained compound of less than 30 min. A chromatogram of MnDPDP standard spiked with impurity standards is presented in Fig. 2.

Some HPLC columns types gave significant on-column oxidation of Mn(II) to Mn(III) and required a column temperature of less than 10°C

in order to minimise this oxidation. The selected column had minimal on-column oxidation at 25°C. However, some columns were found to give an elevated baseline between the Mn(III)DPDP and the MnDPDP peaks due to oxidation when tested for the first time. This baseline elevation was eliminated or reduced by injecting repeatedly a MnDPDP SS standard solution overnight. Fig. 2 presents the chromatograms obtained using an average column.

3.2. Selectivity

MnDPMP, MnDPDP-MOA, Mn(5-methyl)-DPMP, Mn(III)DPDP and MnDPDP were resolved and separated from unidentified degradation products observed in stressed drug substance and stressed pharmaceutical formulation.

3.3. Linearity

The linearities were tested in the following intervals: MnDPDP SS, 0.30–0.50 mg/ml; MnDPMP SS, 0.0002–0.010 mg/ml; MnDPDP-MOA SS, 0.0006–0.0050 mg/ml; Mn(5-methyl)-DPMP SS, 0.0003–0.0016 mg/ml; Mn(III)DPDP SS, 0.0003–0.0020 mg/ml. Correlation coefficients (r) were 0.9993, 0.9998, 0.9958, 0.9961, and 0.9894, respectively. Factors of curvature (n; $Y = a + bX^n$) were in the range 0.99–1.03. Linearity was thus found for all compounds tested.

3.4. Precision and accuracy

3.4.1. Assay for MnDPDP SS

A repeatability study (intralaboratory precision; one day, one operator) revealed estimated relative standard deviations (RSDs) of 0.4–0.5% (determined in solutions of 0.30, 0.40 and 0.50 mg/ml with six replicates at each level). The accuracy (assay values relative to sample concentrations based on weight) was estimated as 98.9–99.7%.

The intermediate precision (intralaboratory precision; several days, two instruments, several operators) was performed by regression analysis of stability study data. The RSDs were estimated as 0.4% ($N = 33$) for drug substance and 0.7% ($N = 36$) for pharmaceutical formulation.

3.4.2. Quantification of impurities

The results of testing of repeatability and accuracy (estimated from spike recoveries from a matrix) for MnDPDP-related impurities are presented in Table 1. As shown in the table, the repeatability of the method was good, and the accuracies were acceptable for these low levels of impurities.

The intermediate precision was estimated for Mn(5-methyl)-DPMP in drug substance and MnDPMP in drug product. The RSDs were estimated as 6.1% ($N = 24$) for Mn(5-methyl)-DPMP at a concentration of 0.27% area in drug substance, and 5.1% ($N = 36$) for MnDPMP in the

Table 1
Repeatability ($N = 6$) and accuracy for impurities in mangafodipir trisodium drug substance and pharmaceutical formulation.

Compound[a]	Amount added (mg/ml)	Peak area (% area)	RSD (%)	Accuracy (%)
Mn(III)DPDP SS	0.0003	0.13	12.1	104
	0.0010	0.31	12.6	103
	0.002	0.56	6.2	102
MnDPMP SS	0.0002	0.05	16.0	90
	0.004	1.11	2.2	101
	0.008	2.23	1.3	102
MnDPDP-MOA SS	0.0006	0.15	5.4	123
	0.003	0.67	2.2	106
	0.004	1.04	2.8	106
Mn(5-methyl)-DPMP SS	0.0003	0.07	10.4	–
	0.0008	0.18	2.6	108
	0.0012	0.29	1.6	105

[a] Refer to Fig. 1.

Table 2
Limits of detection (LOD) and limits of quantification (LOQ) for impurities in mangafodipir trisodium drug substance of pharmaceutical formulation

Compound[a]	LOD			LOQ		
	ng	μg/ml	% area	ng	μg/ml	% area
Mn(III)DPDP SS	1	0.1	0.10	3	0.3	0.13
MnDPMP SS	1	0.1	0.03	2	0.2	0.05
MnDPDP-MOA SS	2	0.2	0.04	6	0.6	0.15
Mn(5-methyl)-DPMP SS	1	0.1	0.03	3	0.3	0.07

[a] Refer to Fig. 1.

concentration range 0.5–0.8% area in pharmaceutical formulation.

3.5. Limits of detection and quantification

The limits of detection (LODs) and limits of quantification (LOQs) were defined as amounts of substance producing peak heights corresponding to three and ten standard deviations of blank fluctuations. The standard deviation of the blank was estimated as the peak-to-peak noise divided by five, and the peak-to-peak noise was measured within a time interval corresponding to 20 times peak width at half height [7]. The estimated LODs and LOQs are presented in Table 2. The LOQs for impurities related to MnDPDP SS were acceptable for quality control of these compounds in drug substance and pharmaceutical formulation.

3.6. Response factors

The UV spectra for MnDPDP SS and related compounds are similar, with molar absorptivities at absorption maxima (range, 308–319 nm) of the same order of magnitude (range, 8.8×10^3–13.4×10^3 cm^{-1} M^{-1}). The relative molar response factors (peak area response relative to MnDPDP SS) using the present method were found to be 1.07 for Mn(III)DPDP, 1.00 for MnDPMP, 0.92 for Mn(5-methyl)-DPMP, and 0.80 for MnDPDP-MOA. The relative responses thus justified the use of area normalisation for the determination of these impurities in drug substance, as well as in pharmaceutical formulation.

4. Conclusion

Mangafodipir trisodium and related impurities were determined by single-point calibration and area normalisation by UV respectively, using a simple isocratic HPLC method utilising a polymeric reverse-phase column at a high pH and tetrabutylammonium ion-pair reagent. The analytical properties of the method described were found suitable for quality control and stability testing of MnDPDP SS with respect to assay as well as determination of the related impurities MnDPMP, MnDPDP-MOA, Mn(5-methyl)-DPMP and Mn(III)DPDP in drug substance and pharmaceutical formulation.

References

[1] C.G. Torres, B. Lundby, A. Tufte Sterud, S. McGill, P.B. Gordon, H. Strand Bjerknes, Acta Radiol. 38 (1997) 631–637.
[2] S.M. Rocklage, W.P. Cacheris, S.C. Quay, F.E. Hahn, K.N. Raymond, Inorg. Chem. 28 (1989) 477–485.
[3] B. Tirkkonen, A. Aukrust, E. Couture, D. Grace, Y. Haile, K.M. Holm, H. Hope, Å. Larsen, H. Sivertsen Lunde, C.E. Sjøgren, Acta Radiol. 38 (1997) 780–789.
[4] P. Janos, J. Chromatogr. A 719 (1996) 457–461.
[5] P. Janos, J. Chromatogr. 635 (1993) 257–263.
[6] J.J. Lucena, P. Barak, L. Hernández-Apaolaza, J. Chromatogr. A 727 (1996) 253–264.
[7] J.P. Foley, J.G. Dorsey, Chromatographia 18 (1984) 503–511.