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Determination of dysprosium in monkey serum by inductively-coupled plasma atomic emission spectrometry (ICP-AES) after the administration of Sprodiamide Injection, a new contrast medium for magnetic resonance imaging*

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Abstract: Sprodiamide Injection (S-043 Injection, Nycomed Salutar; WIN 59080, Sterling Winthrop) is a magnetic susceptibility-based MRI contrast agent which contains 500 mM dysprosium diethylenetriaminepentaacetic acid bis(methylamide) (DyDTPA-BMA), and 25 mM caldiamide sodium (CaNaDTPA-BMA). A study was conducted to evaluate clearance of drug in cynomolgus monkeys. Eighteen cynomolgus monkeys, divided into three groups of six animals each, were administered Sprodiamide Injection intravenously at dose levels of 0.25, 0.5 and 2.5 mmol kg^{-1}, respectively. The concentration of dysprosium in serum was determined in a monkey serum–hydrochloric acid matrix by inductively-coupled plasma atomic emission spectrometry (ICP-AES). The ICP-AES method was demonstrated to be valid for sensitivity, precision, accuracy, and specificity. The dynamic range was linear from 0 to 50 μg ml^{-1} and the limit of quantification was 24 ng ml^{-1}. The measured dysprosium concentration in monkey serum ranged from 0 to 339 μg ml^{-1} for the 0.25 mmol kg^{-1} Sprodiamide Injection dose group, from 0 to 633 μg ml^{-1} for the 0.5 mmol kg^{-1} and from 0 to 2920 μg ml^{-1} for 2.5 mmol kg^{-1} dose groups. Dysprosium was not detected after 480 min in any of the serum samples from the 0.25 and 0.5 mmol kg^{-1} dose groups after the administration of Sprodiamide Injection. All the monkeys in the 2.5 mmol kg^{-1} dose group, with one exception, required 720 min for clearance of the drug from the serum. The drug was completely cleared from serum in all monkeys within 24 h.

Keywords: *Sprodiamide Injection; S-043 Injection; dysprosium; serum; ICP-AES; bioanalysis; method validation; MR, contrast agent.*

Introduction

Magnetic resonance imaging (MRI) using magnetopharmaceutical contrast agents has provided diagnostic utility by enhancing differentiation between normal and abnormal tissues and blood flow *in vivo* [1–3]. Sprodiamide Injection (Fig. 1) is a potential magnetic susceptibility perfusion contrast agent useful in visualizing image intensity changes during the vascular transit of the drug [1, 4]. The purpose of this study was to develop and validate a method used to measure the serum dysprosium concentration in monkeys administered Sprodiamide Injection and to obtain pharmacokinetic parameters.

A common method used to measure the level of metals in biological fluid is to digest the sample with acids, or dilute with water, and analyse the resulting solution by atomic absorption or emission techniques [5, 6]. However, the process of sample digestion may cause low recovery and result in a higher dilution of sample. Due to the limited amount of sample available in the present study (<0.25 ml each), a simple method was developed for the analysis of the dysprosium content in serum by directly dissolving the serum sample in a 1 N HCl aqueous solution and analysing the sample by ICP-AES. The ICP system demonstrated improved stability when analysing the dissolved samples in acidic medium compared to water. The method reported herein was found to be specific, sensitive, precise and accurate for determination of the dysprosium content in a serum–1 N HCl (1:100, v/v) matrix in a range from 24 ng ml^{-1} to 50 μg ml^{-1}. The procedure was applied to measure the dysprosium level in 232 monkey serum samples.

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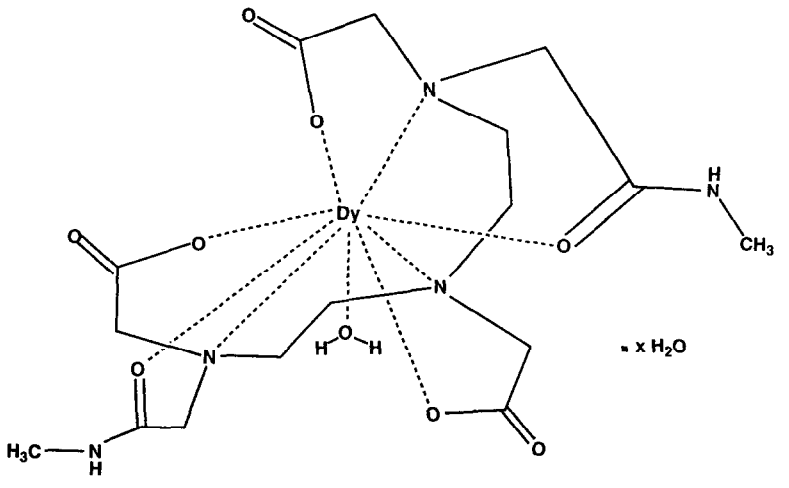

Figure 1
Chemical structure of DyDTPA-BMA, the active ingredient of Sprodiamide Injection.

Experimental

Reagents and materials

Dysprosium standard ($DyCl_3$ in 10% HCl) was obtained from the National Institute of Standards and Technology (NIST). Iron standard ($FeCl_3$ in 10% HCl), traceable to NIST, was obtained from Aldrich. Monkey control serum was obtained from Sigma. Diethylenetriaminepentaacetic acid bis(methylamide), DTPA-BMA, was obtained from Nycomed Imaging AS (Oslo, Norway).

Instrumentation and ICP-AES conditions

A JY-24 ICP system from Instrument SA was used for all measurements. The dysprosium and reference emission wavelengths were 353.171 and 193.031 nm, respectively. The radio-frequency power was 1100 watts. The argon pressure was 80 psi and argon flow was 12 l min^{-1} for plasma gas, 0.2 l min^{-1} for auxiliary gas and 0.4 l min^{-1} for the nebulizer gas. The peristatic pump rate was 1.35 ml min^{-1}. The signal attenuation was established by aspirating the highest concentration of standard solution (50 μg ml^{-1}) into the plasma and running the auto-attenuate mode.

Collection of biological samples

Eighteen cynomolgus monkeys, divided into three groups of six animals each (three males and three females), were administered Sprodiamide Injection intravenously at dose levels of 0.25, 0.5 and 2.5 mmol kg^{-1}, respectively. Thirteen blood samples were collected from each monkey (0.5 ml whole blood to provide 0.25 ml serum). The samples were collected before administration, at 3, 6, 9, 15, 30, 45, 60, 120, 240, 480 and 720 min, and 24 h after administration of Sprodiamide Injection. Two samples, at 3 and 15 min post-dose, were not obtained from one of the monkeys (monkey I-1). A total of 232 monkey serum samples were collected for dysprosium determinations. The intravenous administration of Sprodiamide Injection to cynomolgus monkeys and serum sample collection were performed by the New Mexico Primate Research Laboratory, NMSU.

Preparation of standards

A stock solution of dysprosium was prepared at 250 μg ml^{-1} in 1 N HCl aqueous solution. Appropriate dilutions of the stock were made with 1 N HCl. The spiking solutions were used to prepare working standards in a monkey serum–1 N HCl (1:100, v/v) matrix at dysprosium concentrations of 0, 5, 25 and 50 μg ml^{-1}. Pools of quality control (QC) sample of 100 ml each were prepared at a final concentration of 25 μg ml^{-1}.

Sample preparation for method validation

Appropriate dilutions of the standard stock were made with 1 N HCl and the spiking solutions were used to prepare spiking samples in a monkey serum–1 N HCl (1:100, v/v) matrix at dysprosium concentrations of 0, 0.004, 0.008, 0.012, 0.024, 1, 5, 10, 25, 35 and 50 μg ml^{-1}. A blank monkey serum–HCl solution containing DTPA-BMA was prepared. Iron solutions (50 μg ml^{-1}) in 1 N HCl

with and without spiked 5 μg ml^{-1} of dysprosium were also prepared.

Sample preparation

Aliquots of 50 μl of each monkey serum were diluted to 5 ml with 1 N HCl prior to ICP analysis. Samples were not subjected to conventional heat treatment or microwave digestion.

Results and Discussion

Linearity and quantification limit

A linear relationship between concentration and atomic emission response of dysprosium was observed. The dynamic linear range was from 0 to 50 μg ml^{-1} of dysprosium. The correlation coefficient was greater than 0.999.

The detection limit was determined using a blank solution and confirmed with a spiked solution at 12 ng ml^{-1}. The limit of quantification (LOQ), found to be 24 ng ml^{-1}, twice the detection limit (LOD).

Precision and accuracy

The intra-day precision and accuracy was assessed by analyses of multiplicate spiked samples at four different concentrations, 0.024, 5, 25 and 50 μg ml^{-1}. The range of precision (Table 1) was from 0.62% (50 μg ml^{-1} dysprosium) to 1.80% (0.024 μg ml^{-1} dysprosium) RSD. The mean precision was 1.13% RSD. The accuracy expressed as a percentage of nominal concentration of the four dysprosium spiked samples is summarized in Table 2. The mean accuracy, 98.4%, ranged

Table 1
Intra-day precision of dysprosium in monkey serum–HCl matrix

Nominal conc. (μg ml^{-1})	*n*	Mean conc. found (μg ml^{-1})	RSD (%)
0.024	10	0.0239	1.80
5	10	4.92	0.90
25	10	25.30	1.20
50	10	50.21	0.62

Table 2
Intra-day accuracy of dysprosium in monkey serum–HCl matrix

Nominal conc. (μg ml^{-1})	*n*	Mean ± RSD (%) conc. found (μg ml^{-1})	% Recovery*
0.024	6	0.0234 ± 1.93	97.5
5	6	4.89 ± 0.41	97.8
25	6	24.61 ± 0.51	98.4
50	6	49.85 ± 1.10	99.7
Mean			98.4

* % Recovery = (found conc. × 100)/(nominal conc.).

Table 3
Inter-day precision and accuracy of dysprosium in monkey serum–HCl matrix

Sample ID	QC sample at 25 μg ml^{-1} *n*	RSD (%)	% Recovery*
QC 1	14	1.25	99.32
QC 2	30	1.39	99.92
QC 3	25	1.56	99.52
QC 4	18	1.12	99.84
Mean			99.65

* % Recovery = (found conc. × 100)/(nominal conc.).

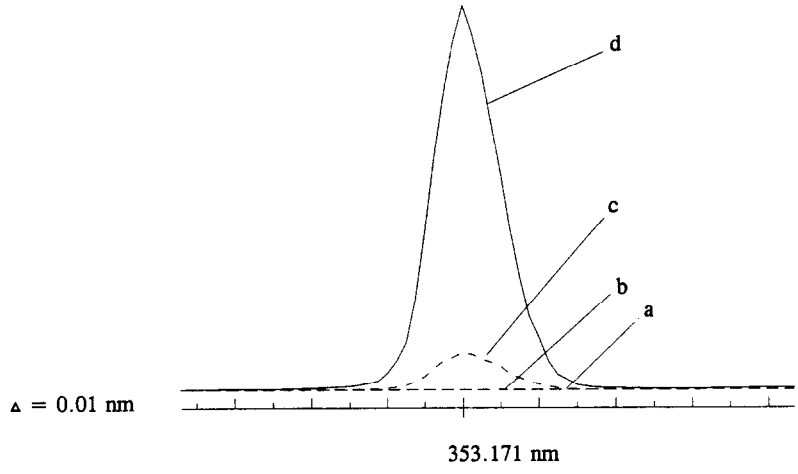

Figure 2
Specificity: atomic emission spectra of (a) serum blank, (b) iron standard (50 μg ml^{-1}), (c) iron standard (50 μg ml^{-1}) spiked with dysprosium (5 μg ml^{-1}) and (d) dysprosium standard (50 μg ml^{-1}).

Table 4
Dysprosium found ($\mu g\ ml^{-1}$) in monkey serum following administration of Sprodiamide Injection

Animal ID	Pre-dose	Time (min) 3	6	9	15	30	45	60	120	240	480	720	1440
0.25 mmol kg^{-1} dose group													
I-1*	0.0	n/a	49.8	68.1	n/a	99.4	94.7	92.9	56.4	18.3	0.0	0.0	0.0
I-2	0.0	259.7	217.8	186.9	170.0	106.3	78.1	67.1	29.4	5.0	0.0	0.0	0.0
I-3	0.0	339.1	312.1	256.0	168.0	123.9	95.7	80.7	39.3	9.9	0.0	0.0	0.0
I-4	0.0	306.3	241.0	209.9	164.8	104.1	75.9	58.1	24.3	4.1	0.0	0.0	0.0
I-5	0.0	303.6	242.3	200.7	160.2	107.2	80.6	62.1	23.8	6.6	0.0	0.0	0.0
I-6	0.0	256.2	217.6	191.2	186.9	133.0	110.9	81.0	97.5	9.7	0.0	0.0	0.0
Mean (n = 5)	0.0	293.0	246.1	209.0	170.0	114.9	88.2	69.8	42.9	7.1	0.0	0.0	0.0
RSD (%)	n/a	11.9	15.7	13.3	6.0	11.2	16.9	15.1	72.7	38.0	n/a	n/a	n/a
0.5 mmol kg^{-1} dose group													
II-1	0.0	499.3	396.5	325.8	274.4	191.7	127.5	94.6	34.2	6.3	0.0	0.0	0.0
II-2	0.0	568.7	472.5	375.6	311.7	206.9	140.3	111.9	38.7	7.5	0.0	0.0	0.0
II-3	0.0	588.4	298.3	430.5	349.1	231.9	162.2	118.7	45.6	16.0	0.0	0.0	0.0
II-4	0.0	632.7	499.1	452.2	392.9	255.6	214.6	159.8	53.8	17.9	0.0	0.0	0.0
II-5	0.0	566.1	407.5	376.7	316.4	232.7	156.7	138.3	64.2	17.1	0.0	0.0	0.0
II-6	0.0	519.5	420.6	342.5	294.4	218.7	152.3	111.7	46.8	16.9	0.0	0.0	0.0
Mean (n = 6)	0.0	562.4	415.7	383.9	323.2	222.9	158.9	122.5	47.2	13.6	0.0	0.0	0.0
RSD (%)	n/a	8.5	16.8	12.8	13.1	9.8	18.9	18.8	22.8	38.6	n/a	n/a	n/a
2.5 mmol kg^{-1} dose group													
III-1	0.0	2202.1	1966.3	1720.5	1434.5	936.0	720.7	375.1	222.2	56.2	11.5	0.0	0.0
III-2	0.0	2471.2	1921.5	1651.3	1344.4	868.5	598.0	430.9	199.2	33.9	2.5	0.0	0.0
III-3	0.0	2920.0	2085.0	2015.7	1781.5	1566.7	1072.2	911.7	693.5	275.8	79.4	33.8	0.0
III-4	0.0	2087.4	1786.2	1575.5	1411.6	926.6	758.4	585.8	218.4	115.0	2.7	0.0	0.0
III-5	0.0	2261.9	1938.8	1527.2	1276.5	931.6	778.8	603.2	281.0	78.2	5.8	0.0	0.0
III-6	0.0	2546.4	2199.7	2083.0	1914.7	1388.2	1093.7	870.7	441.6	173.6	14.0	0.0	0.0
Mean (n = 6)	0.0	2414.9	1983.1	1762.2	1527.2	1102.9	837.0	629.6	342.7	122.1	19.3	5.6	0.0
RSD (%)	n/a	12.4	7.2	13.2	16.9	26.9	24.0	35.1	56.5	73.6	154.5	245.0	n/a

n/a = not available.
*Excluded from pharmacokinetic analysis.

from 97.5 to 99.7%, and the mean RSD, 0.99%, ranged from 0.41 to 1.93%.

The inter-day precision and accuracy (Table 3) was determined by repeatedly analysing quality control samples at 25 μg ml^{-1} of dysprosium concentration. The mean precision and accuracy ranged from 1.12 to 1.56% RSD, and 99.32 to 99.92% of the nominal concentration, respectively.

Specificity

Spectral and background interferences of monkey serum were investigated at the dysprosium emission wavelength, 353.171 nm. The emission spectra in Fig. 2 shows no interferences between dysprosium and the monkey serum matrix or iron, the major metal in the blood pool.

Analysis for the monkey serum samples

Table 4 summarizes the dysprosium concentration found in monkey serum following administration of Sprodiamide Injection. The measured dysprosium concentrations ranged from 0 (below LOQ) to 339 μg ml^{-1} for the 0.25 mmol kg^{-1} Sprodiamide Injection dose group, from 0 to 633 μg ml^{-1} for the 0.5 mmol kg^{-1} and from 0 to 2920 μg ml^{-1} for 2.5 mmol kg^{-1} dose groups. Dysprosium was not detected after 480 min in any of the serum samples from the 0.25 and 0.5 mmol kg^{-1} dose groups after the administration of Sprodiamide Injection. All the monkeys in the 2.5 mmol kg^{-1} dose group, with one exception (monkey III-3), required 720 min for clearance of the drug from the serum. The drug was completely cleared from serum in all monkeys within 24 h. Data obtained from monkey I-1 was not averaged in that group due to sample collection problems. Figures 3, 4 and 5 show the time-dependent profile of dysprosium concentration in serum for each dose group following the administration of Sprodiamide Injection.

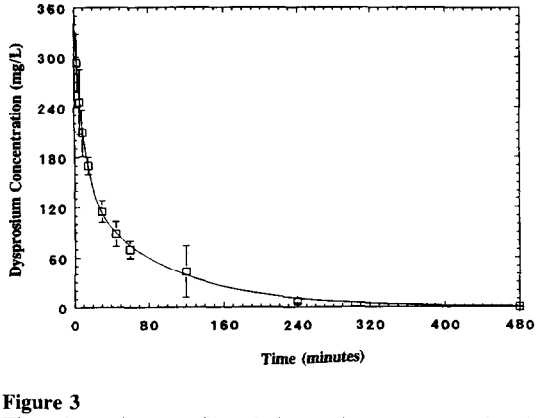

Figure 3
Time-dependent profile of dysprosium concentration in serum from the dose group following administration of 0.25 mmol kg^{-1} Sprodiamide Injection.

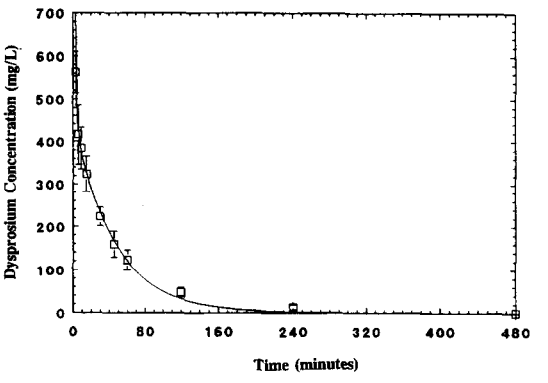

Figure 4
Time-dependent profile of dysprosium concentration in serum from the dose group following administration of 0.5 mmol kg^{-1} Sprodiamide Injection.

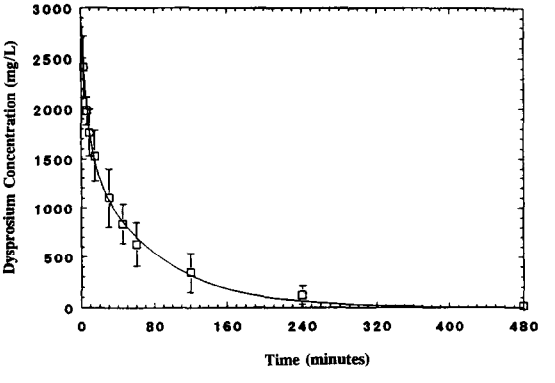

Figure 5
Time-dependent profile of dysprosium concentration in serum from the dose group following administration of 2.5 mmol kg^{-1} Sprodiamide Injection.

Detailed analysis of the data to obtain the pharmacokinetic parameters of Sprodiamide Injection in monkeys has been described elsewhere [7].

Conclusions

The method described is a convenient way to use ICP-AES to determine dysprosium concentration in monkey serum for small sample volumes (<0.25 ml). The method has been shown to be sensitive, specific, precise and accurate. Heat assisted digestion of serum samples was not required. The high per cent nominal dysprosium concentration found

(>99.0%) with low RSD (<2%) for the QC samples demonstrate system stability and accuracy of the assay. The level of dysprosium in 232 serum samples was able to be measured using the described method.

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