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Stability and sterility of meglumine gadoterate injection repackaged in plastic syringes

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Abstract

The analytical and microbiological stability of meglumine gadoterate (Dotarem®) repackaged in polypropylene syringe for 3 months at either +4°C or room temperature was studied. For analytical study: six polypropylene syringes (20 ml) were filled with 15 ml of meglumine gadoterate. Three syringes were stored at 4 ± 2°C and three at 25 ± 2°C, all syringes were kept upright and protected from daylight. Samples were taken on days 0, 6, 14, 30, 45, 60, 75 and 90. Meglumine gadoterate and its degradation product (free Gd^{3+}) concentrations were obtained using a specific HPLC assay. Osmolality and pH determination were made on days 0, 14, 45 and 90. For microbiological study: 28 plastic syringes (5 ml) were filled with 2.5 ml of meglumine gadoterate. Syringes were stored at 25 ± 2°C and protected from daylight. At each day of analysis (0, 15, 35, 45, 60, 75 and 90), four syringes were tested as described in European Pharmacopoeia. After 90 days the concentration of gadoterate remained unchanged and no free Gd^{3+} were detected. The injectable solution of this gadolinium contrast agent was sterile according to European Pharmacopoeia guidelines. The meglumine gadoterate repackaged in polypropylene syringe was stable for 3 months at all the temperatures studied.

Keywords: Gd–DOTA; Pediatric; MRI; Syringes; Stability

1. Introduction

Paramagnetic lanthanide chelates are effective Magnetic Resonance Imaging (MRI) agents (Runge et al., 1989, 1990). Meglumine gadoterate (Dotarem®) is made of a gadolinium complex of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (27.93 g/100 ml) and meglumine (9.76 g/100 ml). It is a widely used gadolinium based MRI contrast agent licensed for pediatric use in France from 1 month of age (Vidal, 1998). In pediatric patients, the usual dose administered is 0.2 ml/kg. Meglumine gadoterate is commer-

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cially available on the market as 5- and 10-ml glass syringes and vials only. This meglumine gadoterate presentation is not intended for newborn or infants less than 25 kg leading to a 30% loss of drug because a single dose is used and the remainder is discarded (Gonçalves-Martins, 1997). We proposed to the radiologist staff preparation of pre-filled syringes for these specific patients. Since no data was available about the compatibility of gadolinium chelates repackaged into a plastic syringe, a stability study was necessary.

Physico-chemical stability of a gadolinium MRI contrast agent is particularly critical, as toxicity of these compounds are directly related to free Gd^{3+} (Cacheris et al., 1990; Berha-Miellet et al., 1995). A MRI contrast agent must remain intact to minimize the concentration of free metal ions and ligand. Release of Gd^{3+} from the 1,4,7,10 tetraacetic ligand (DOTA) could theoretically be induced by some components of a plastic syringe (i.e. Ca^{2+} stearate is a polypropylene additive) via a transmettalation reaction because the ionic radius of Gd^{3+} (1.02 Å) is very similar to that of calcium (0.99 Å). This transmetallation mechanism is acknowledged in vivo only when Ca^{2+} is largely in excess versus Gd^{3+} (Crooks et al., 1997). It has been shown that free Gd^{3+} could disturb calcium dependant functions such as molecular contraction and neurotransmission (Wolf and Frobben, 1984). A human case report of persistent enhancement of a brain mass for 9 days after contrast medium suggested dechelation in vivo (Tien et al., 1989). Other potential interactions between meglumine gadoterate and a plastic syringe include: adsorption of meglumine gadoterate onto the syringe inner walls and diffusion of syringe components into the meglumine gadoterate solution.

In order to determine the compatibility of meglumine gadoterate solution repackaged into a plastic syringe we carried out a physico-chemical and microbiology study for 3 months. The aims of the present study required a specific analytical method to simultaneously measure Gd–DOTA and free gadolinium.

2. Materials and methods

2.1. Samples preparation

Ten vials of meglumine gadoterate (Laboratoire Guerbet, Roissy-Charles-de-Gaulle, France) were manipulated under a laminar flow cabinet located in a clean room (class A), and pooled in a sterile glass container. The solution was then packed into polypropylene syringes and an aliquot was repackaged into a sterile glass vials to make the control solution. Single-use polypropylene syringes 20 ml were filled with a total of 15 ml of meglumine gadoterate. The volume of sampling (15 ml) was determined to have enough meglumine gadoterate throughout the stability study. On each day of analysis, 0.2 or 3.5 ml were withdrawn from the sample depending on the different analysis to be done, e.g. HPLC analysis (0.2 ml) and/or osmolality and pH measurements (3.5 ml). We considered that it was more important to get sampling from the same syringe over the stability study. These considerations led us to dispense 15 ml in the syringe. For the HPLC analysis and/or osmolality and pH measurements, samples withdraw were not performed under aseptic conditions.

All repackaged plastic syringes tips were aseptically capped with a sterile syringe tip cap.

2.2. Protocol

Among the six syringes (S) prepared for the analytical part of the study, S_1, S_2, S_3 were stored at 4 ± 2°C while S_4, S_5 and S_6 were kept at 25 ± 2°C. All syringes were stored upright with hub downwards, to prevent contact between meglumine gadoterate solution and the rubber plunger seal, a volume of 1 ml of air was pulled to prevent this contact. All syringes were protected from daylight by a opaque bags. Sampling times scheduled at different days (D) were D_0, D_6, D_{14}, D_{30}, D_{45}, D_{60}, D_{75} and D_{90}.

After shaking smoothly the syringe, 0.2–3.5 ml were collected, depending on analysis to be made.

The following parameters were performed each day of analysis:

- Visual aspect of the solution,

- The integrity of the syringe: absence of leak, fitting of the piston head within the syringe,
- Measurement of Gd–DOTA and Gd^{3+} on 1/100 dilution for each three syringe,
- Checking of the absence of free Gd^{3+} detected as Gd–CDTA chelate on a non diluted sample from the syringes S_1 to S_6.

A 1/100 dilution was made before measurements of Gd–DOTA into samples to avoid the inner filter effect usually observed when dealing with luminescence detection. Regarding Gd^{3+} measurements, dilution was inappropriate as the lanthanide ion, degradation product of the complex, was expected as traces only if present.

All measurements were performed in triplicate.

On D_0, D_{14}, D_{45} and D_{90} sampling times only, osmolality and pH were also measured.

2.3. Methods

To monitor simultaneously Gd–DOTA concentration and free Gd^{3+} as a Gd–CDTA chelate, an HPLC method using a time resolved luminescence (TRL) detector was used as described previously (Moutiez et al., 1997). Gadolinium belongs to the lanthanide group that exhibits particular spectroscopic characteristics, e.g. a long emission lifetime, a fine emittive structure and an emission drastically enhanced by chelation. The long intrinsic lifetime of lanthanides can be exploited by time resolved measurements. Moutiez tested several EDTA like agents, in order to be able to detect Gd^{3+} as Gd–EDTA like complex. Among these, CDTA exhibited the best-cost efficacy ratio. Some minor adaptations were made: 2.5% of acetonitrile were used instead of 2% in the mobile phase, the excitation and emission slits and the detector were different as described below.

A Beckman metering pump 126® (Beckman, Gagny, France), equipped with a Rheodyne 7125® (Cotati, USA) injection valve (20-μl loop) was used to perform HPLC experiments. The 150 × 4.6-mm column, packed with a Chromospher® C8 stationary phase (Chrompack, Les Ulis, France), was chosen. The time resolved luminescence detector used was a LS 5 luminometer (Perkin Elmer, Palo Alto, CA). The wavelength settings were $\lambda_{exc} = 274$ nm and $\lambda_{em} = 315$ nm. Excitation and emission slits were 5 and 10 nm, respectively.

The mobile phase consisted of (α,α,α Tris hydroxymethy) aminomethane (Sigma Chemical, St Louis, USA)–hydrochloric acid 0.2 M buffer (Labosi, Maurepas, France) (pH 7.6)/acetonitrile (Carlo Erba Reagenti, Val de Reuil, France) 97.5:2.5 (v/v). The mobile phase flow rate was 0.6 ml/min. Retention times were 3.4 and 6.3 min Gd–DOTA and Gd–CDTA, respectively.

The method was validated according to SFSTP (Sociéte Française des Sciences et Techniques Pharmaceutiques) guidelines (Caporal-Gauthier et al., 1992). These guidelines are quite similar to the ICH requirements.

The meglumine gadoterate stock solution was 0.5 mmol/ml. Five standard solutions (1.75, 3.5, 5, 7 and 10 μmol/ml) were prepared by diluting the stock solution with water. The Gd^{3+} (Aldrich Chem Co, Milwaukee, USA) chelate CDTA (Sigma Chemical, St Louis, USA) stock solution was prepared by wheighing an appropriate amount of the chemicals and diluted with Tris–HCl buffer. The concentration of the solution was 10^{-2} M. Five standard solutions (0.1, 0.2, 0.3, 0.4 and 0.5 μmol/ml) were prepared. Standard curves constructed for the assay were linear ($R^2 = 0.9986$ for meglumine gadoterate and $R^2 = 0.9974$ for Gd–CDTA). Intraday coefficients of variation were 2.5 and 1.2% respectively for 3.5 and 7 μmol/ml Gd–DOTA solution. Intraday coefficients of variation were 5.0 and 4.3% respectively for 0.2 and 0.4 μmol/ml Gd–CDTA solution. Interday coefficients of variation were 3.0 and 1.5% respectively for 3.5 and 7 μmol/ml Gd–DOTA solution. Interday coefficients of variation were 6.4 and 2.3% respectively for 0.2 and 0.4 μmol/ml Gd–CDTA solution.

Standards were run before and after unknown samples as described previously (McDowall, 1998).The stability-indicating nature of the assay was determined by accelerating the degradation of meglumine gadoterate. One millilitre of meglumine gadoterate was mixed with 11.8 N hydrochloric acid adjusted to pH 1 or with 10.8 N sodium hydroxide adjusted to pH 14. A third mixture was kept at the initial pH 6.6. All three mixtures were placed into a drying oven at 60°C

for 12 h. Each solution was then processed as previously described. After the degradation, peak of Gd–DOTA was reduced and a Gd^{3+} peak was identified as a Gd–CDTA peak.

All concentrations, which were expressed as a percentage of the original concentration (100%), were the mean of triplicate aliquots.

The limit of detection was obtained by use of the slope and the standard deviation of the intercept from six calibrations graphs determined by linear regression line as defined by ICH.

For pH measurements, a 300pH meter (Beckman, Gagny, France) was used. Calibration was done with a pH 4 and a pH 7 solution (Prolabo, Fontenay sous Bois, France). For osmolality measurements a Advanced osmometer 3D3 (Radiometer Tacussel, Neuilly-Plaisance, France) was used, calibration was done with a 1500 mosm/kg solution. (Advanced Instruments Inc., Massachusetts, USA).

2.4. Microbiology study

On D_0, seven vials of meglumine gadoterate were used to fill with 2.5-ml 28 single-use polypropylene syringe of 5 ml under a laminar flow cabinet located in a clean room (class A). At D_0, D_{15}, D_{35},D_{45}, D_{60},D_{75} and D_{90}, four syringes were tested as described in both European Pharmacopoeia (1997) and US Pharmacopeia (1999) for the control of sterile preparation including validation test for bacteriostasis and fungistatis to ensure that any material does not interfere with the sterility test. A closed system, Steristest R (Millipore, Saint-Quentin-en-Yvelines, France) was used.

2.5. Statistical treatment

Validation of the HPLC method and statistical treatment of data were done using Sigma-Stat® software (Version 1.0, Jandel Corporation, Germany). Differences regarding Gd–DOTA concentration in syringes were analyzed using a one way ANOVA test. The level of significance for all comparison was set at $P < 0.05$.

3. Results

Over the 90 days of analysis, all the meglumine gadoterate solutions repackaged in plastic syringes remained clear and limpid. The fitting of the piston head within the syringe was correct after 90 days. No leaks were observed from the syringe. No coloration, precipitation or gas were observed.

According to the manufacturer specifications, the pH must be between 6.5 and 8. Measurements of pH complied with the manufacturer specifications. All osmolality measurements were in the range 1282–1414 mosM, within a 5% range of the target and were considered not to be significant. The target value obtained was 1361.52 ± 8.12 mosM.

Average concentrations of meglumine gadoterate at the different days of analysis are reported in Table 1. As an illustration, chromatograms of standards (meglumine gadoterate and Gd^{3+} chromatographied as Gd–CDTA chelate), of diluted and pure meglumine gadoterate contained syringes are shown in Fig. 1. This figure shows two standards and the material obtained from the syringe during the test. The latter is important and shows there is no free Gd^{3+} (subsequently chelated with CDTA) in the samples.

Microbiological did not reveal growth in any samples over the whole period of time of the study, the sterility test was passed.

4. Discussion

This is, to our knowledge, the first stability study of a MRI contrast agent repackaged in a plastic syringe. No significant differences were observed regarding Gd–DOTA concentration between different days of analysis ($P < 0.05$). There were no statistical differences between S_1 to S_3 and S_4 to S_6, which makes storage possible either at room temperature or in the fridge (ANOVA $P < 0.05$). Owing to the Gd–DOTA formation constant (Cacheris et al., 1990), i.e. $\log K = 25.3$, Gd^{3+} released from the complex is very low. The differences observed are related to the variability of the assay and could have obscured small real

differences. Nevertheless in any non diluted sample of meglumine gadoterate injected into the chromatography, no Gd^{3+} (as Gd–CDTA) peak was observed. This strongly suggests that there are no significant differences after 3 months. The authors proposed a shelf-life of 90 days.

We investigated further to determine which theoretical free Gd^{3+} concentration could be detected by the proposed method.

If we consider the reaction of complex formation:

$$[Gd^{3+}] + [DOTA] \rightleftarrows [Gd\text{–}DOTA]$$

K is given by the following equation:

$$K = \frac{[Gd - DOTA]}{[Gd^{3+}][DOTA]} \quad (1)$$

Because the stoechiometry of [Gd–DOTA] is 1:1 and the value of K is reported in the literature $K = 10^{25}$, Eq. (1) can be written as:

$$K \cong \frac{[Gd - DOTA]}{[Gd^{3+}]^2} \quad (2)$$

and as [Gd–DOTA] corresponds to 10^{-5} M injected pure Dotarem® is analyzed.

Eq. (2) yields to

$$[Gd^{3+}] \cong \sqrt{\frac{[Gd - DOTA]}{K}} = 10^{-15}\ \text{M}$$

According to this demonstration, a concentration of free Gd^{3+} as low as 10^{-15} M would be present in gadoterate meglumine. The HPLC method selected is not sensitive enough to detect such a low level of free Gd^{3+}. However, we attempted to determine the limit of detection (LOD) of free Gd^{3+}, considering the sensitivity limitations of the reported method.

With a determined LOD of 2 nmol of free Gd^{3+}, a percentage of degradation as low as 0.02% of pure or 2% of diluted (1/100) respectively of meglumine gadoterate solution could be detected.

This suggests that the HPLC method used is particularly suitable for pharmaceutical control and in the present case further confirms the stability of the pharmaceutical preparation. This method just required a common fluorimeter that can be set in the 'phosphorescence mode' to record long live luminescence species. It can also be performed with fluorimeter not having this specificity. Therefore, if the measurements are not made in the 'fluorescence mode', sensitivity would not be as good as the one reported in the paper. On the other hand, our manufacturing process, under a laminar flow cabinet was safe, as all samples remained sterile, according to the European Pharmacopoeia (1997) and US Pharmacopeia (1999), during the study.

The risk of Gd^{3+} release was assessed and we concluded that prefilled syringes of meglumine gadoterate are totally innocuous. Nevertheless, hospital pharmacists must consider the inherent toxicity of Gd–DOTA. A recent report of patients who underwent MRI agent administration

Table 1
Drug levels of meglumine gadoterate expressed as average ($n = 3$) percentage of the initial concentration in the syringe at 4 ± 2°C (S_1 to S_3) and at 25 ± 2°C (S_4 to S_6)

	4 ± 2°C			25 ± 2°C		
	S_1	S_2	S_3	S_4	S_5	S_6
D_0	98.0 ± 1.9	98.1 ± 1.4	96.2 ± 0.9	96.9 ± 3.6	101.6 ± 2.0	97.0 ± 4.4
D_6	95.1 ± 0.8	97.6 ± 1.6	97.8 ± 2.5	98.7 ± 0.3	95.9 ± 0.6	96.4 ± 1.8
D_{14}	95.6 ± 0.8	97.2 ± 5.4	95.9 ± 3.5	95.2 ± 2.7	96.0 ± 2.1	96.1 ± 1.4
D_{30}	97.4 ± 3.1	98.5 ± 2.0	98.5 ± 1.9	99.2 ± 2.9	96.2 ± 0.6	97.2 ± 2.3
D_{45}	96.1 ± 2.4	96.9 ± 1.3	96.5 ± 1.1	98.9 ± 3.5	99.1 ± 0.8	95.4 ± 0.8
D_{60}	97.2 ± 0.7	99.0 ± 1.8	97.4 ± 1.4	96.8 ± 6.1	96.4 ± 3.5	97.4 ± 3.6
D_{75}	97.1 ± 0.9	96.4 ± 1.2	98.4 ± 1.6	96.0 ± 1.4	98.1 ± 0.8	98.6 ± 4.1
D_{90}	97.2 ± 1.9	98.4 ± 1.9	101.0 ± 0.5	102.9 ± 0.8	93.8 ± 2.3	95.4 ± 2.1

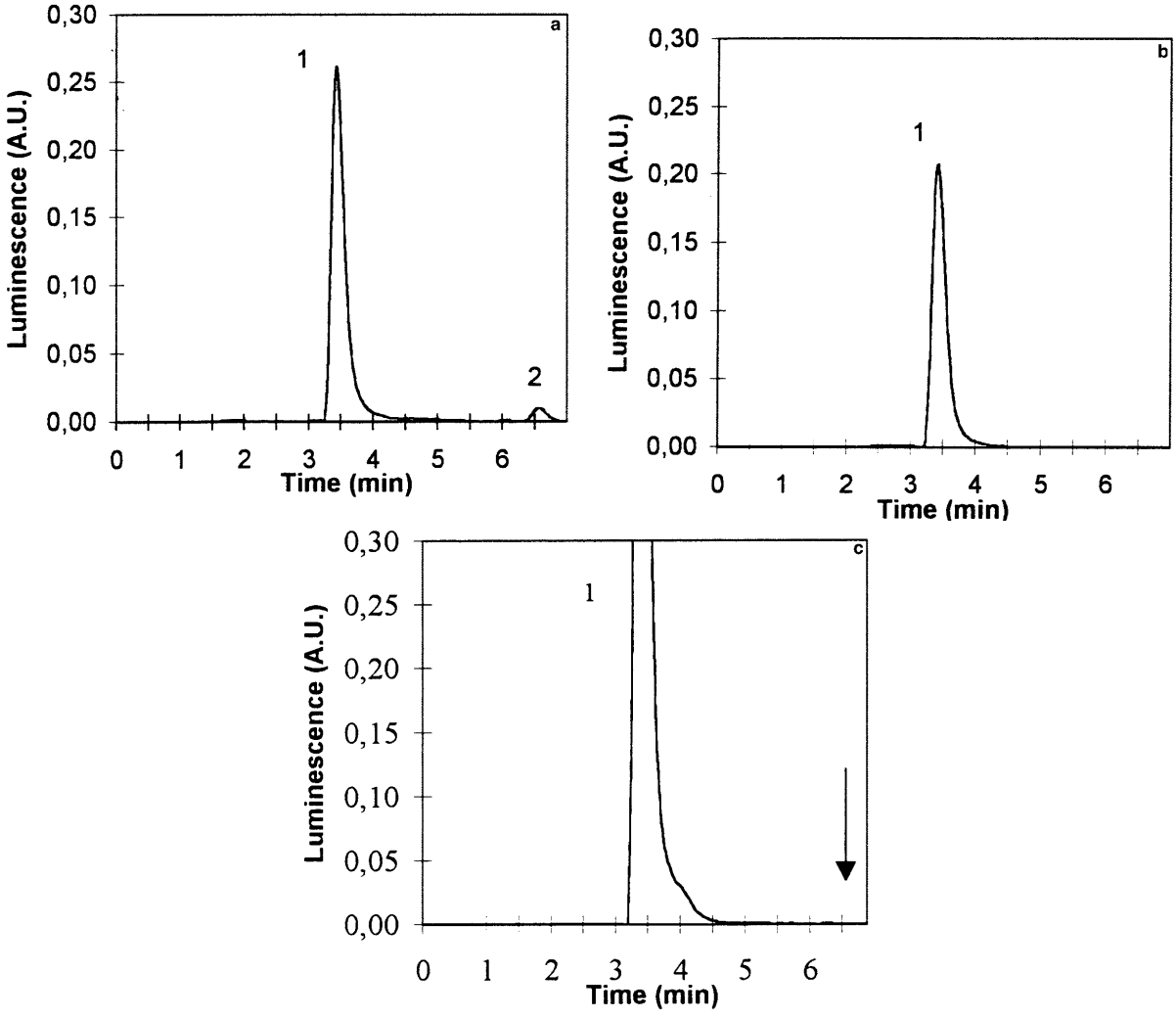

Fig. 1. Typical chromatograms of standard mixture containing 100 nmol of meglumine gadoterate spiked with 6 nmol of free Gd^{3+} (a); of diluted (1/100) (b) and pure (0.5 mmol/ml) (c) meglumine gadoterate contained syringes. Peak 1: meglumine gadoterate; Peak 2: free Gd^{3+} chromatographed as Gd–CDTA chelate.

presented minor adverse effects (Murphy et al., 1996). The authors suggest that as for iodinated contrast media, severe anaphylactic reactions may occur and that personnel must be trained and equipment must be available to treat patients.

The prefilled syringes made at the Pharmacy according to the Good Manufacturing Practices contributes to the reduction of nosocomial infections.

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References

Berha-Miellet, J., Gressier, B., Brunet, C., Dine, T., Luyckx,

M., Cazin, M., Cazin, J.C., 1995. Gadolinium complexs delivered in magnetic resonance imaging: cytotoxicity study and effects of gadoterate meglumine and gadopentate dimeglumine on the oxidative metabolism of human polymorphonuclear neutrophils. Eur. J. Pharm. Biopharm. 41, 354–360.

Cacheris, W.P., Quay, S.C., Rocklage, S.M., 1990. The relationship between thermodynamics and the toxicity of gadolinium complexes. Magn. Reson. Imaging 8, 467–481.

Caporal-Gauthier, J., Nivet, J.M., Algranti, P., Guilloteau, M., Histe, M., Lallier, M., N'Guyen-Huu, Russotto, R., 1992. Guide de Validation Analytique. Société Française des Sciences et Techniques Pharmaceutiques, pp. 205–226.

Crooks, W.J., Choppin, G.R., Rogers, B.E., Welch, M.J., 1997. Evaluation of the in vitro stability of gadolinium (III) polyoxometalates. Nucl. Med. Biol. 24, 123–125.

European Pharmacopoeia, third ed. (1997). Conseil de l'Europe, Strasbourg, pp. 72–76.

Gonçalves-Martins, E., 1997. Contribution à la définition du type et des présentations du produit de contraste idéal en radipédiatrie. Pharmacie, Paris V, France Thèse de Doctorat.

McDowall, R.D., 1998. The next in line. LC-GC Int. 11, 298–302.

Moutiez, E., Prognon, P., Bourrinet, P., Zehaf, S., Dencausse, A., Mahuzier, G., 1997. Time-resolved luminescence as a novel detection mode for the simultaneous HPLC determination of Gadolinium–DOTA and Gd^{3+}. Analyst 122, 1347–1352.

Murphy, K.J., Brunberg, J.A., Cohan, R.H., 1996. Adverse reactions to gadolinium contrast media: a review of 36 cases. Am. J. Roentgenol. 167, 847–849.

Runge, V.M., Carollo, B.R., Wolf, C.R., Nelson, K.L., Gelblum, D.Y., 1989. Gd DTPA: a review of clinical indications in central nervous system magnetic resonance imaging. Radiographics 9, 929–958.

Runge, V.M., Gelblum, D.Y., Pacetti, M.L., Carolan, F., Heard, G., 1990. Gd–HP–DO3 A in clinical MR imaging of the brain. Radiology 177, 393–400.

Tien, R.D., Brasch, R.C., Jackson, D.E., Dillon, W.P., 1989. Cerebral Erdheim–Chester disease: persistent enhancement with Gd–DTPA on MR images. Radiology 172, 791–792.

US Pharmacopeia XXIV (1999). US Pharmacopeial Convention, Rockville, MD, pp. 1818–1823.

Vidal, 1998. Le Dictionnaire, 74th ed, pp. 2071–2072.

Wolf, G.L., Frobben, E.S., 1984. The tissue T1 and T2 response to gadolinium–DTPA injection in rabbits: a potential renal contrast agent for NMR imaging. Invest. Radiol. 19, 324–328.