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Aluminum and Phthalates in Calcium Gluconate; Contribution from Glass and Plastic Packaging

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Aluminum and phthalates in calcium gluconate; contribution from glass and plastic packaging

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Conflict of interest: Robert Yokel is a founder and President of ALKYMOS, Inc. ALKYMOS was developing a device to remove aluminum from calcium gluconate solution. Given the

release of Calcium Gluconate Injection USP in plastic packaging in the US, as cited in this report, which has a concentration of aluminum in the range that was achievable with the ALKYMOS device filtered calcium gluconate from glass-packaged product, ALKYMOS has suspended development of its device. Robert Yokel has no further conflict of interest related to this report. Jason Unrine has no conflict of interest related to this report.

Abstract:

Introduction: Aluminum contamination of parenteral nutrition solutions has been documented for three decades. It can result in elevated blood, bone, and whole body aluminum levels associated with neurotoxicity, reduced bone mass and mineral content, and perhaps hepatotoxicity. The primary aluminum source among parenteral nutrition components is glass-packaged calcium gluconate, in which aluminum concentration the past three decades has averaged ~ 4000 µg/L, compared to < 200 µg/L in plastic container-packaged calcium gluconate. A concern about plastic packaging is leaching of plasticizers, including phthalates, which have the potential to cause endocrine (male reproductive system) disruption and neurotoxicity. Methods: Aluminum was quantified in samples collected periodically over more than two years from three calcium gluconate sources used to prepare parenteral nutrition solutions; two packaged in glass (from France and the US) and one in plastic (from Germany); in a recently released plastic-packaged solution (from the US); and in the two glass containers. Phthalate concentration was determined in selected samples of each product and leachate of the plastic containers. Results: The initial aluminum concentration was ~ 5000 µg/L in the two glass-packaged products and ~ 20 µg/L in the plastic-packaged product, and increased ~ 30, 50 and 100% over 2 years, respectively. The aluminum concentration in a recently released Calcium Gluconate Injection USP was ~ 320 µg/L. Phthalates were not detected in any calcium gluconate solutions or leachates. Conclusion: Plastic packaging greatly reduces the contribution of aluminum to parenteral nutrition solutions from calcium gluconate compared to the glass-packaged product.

What is known:

- Aluminum is a contaminant of parenteral nutrition solutions.
- Aluminum can cause neurotoxicity, reduced bone mass and mineral content, and perhaps hepatotoxicity.
- Glass-packaged calcium gluconate is the primary aluminum source of parenteral nutrition solutions.

What is new:

- As received glass-packaged calcium gluconate provides aluminum in excess of the FDA's recognized level that results in aluminum accumulation associated with central nervous system and bone toxicity.
- Aluminum in calcium gluconate increases over two years of storage.
- Calcium gluconate packaged in the tested plastic containers does not provide aluminum in excess of the FDA level or measurable phthalates (plasticizers with potential to cause male reproductive system disruption and neurotoxicity).

Introduction:

Aluminum contamination of parenteral nutrition solutions has been a documented problem for three decades (1-4), which can result in elevated blood aluminum (5), increased body burden (5, 6), increased bone aluminum (7), neurotoxicity (8), reduced bone mass and mineral content (6, 9), and perhaps hepatotoxicity (10, 11). This led to a US FDA labeling requirement addressing this issue, which concludes that “patients with impaired kidney function, including premature neonates, who receive parenteral levels of aluminum at greater than 4 to 5 [micro]g/kg/day accumulate aluminum at levels associated with central nervous system and bone toxicity” (12). The primary source of aluminum in parenteral nutrition solutions is calcium gluconate, reported to contribute up to 88% vs. ~ 4% from phosphates (13), 88.7% (14), 78% to home parenteral nutrition solutions (15), 81 vs. 9% from sodium phosphate (16), ~ 50% (17), 80% to 98% (18), 80 ± 22% (19), the main source of aluminum (20), and highest amounts were provided by dextrose and calcium gluconate (21). The raw material was identified as one, but not the sole source (22). The aluminum primarily comes from glass packaging (23-26). Aluminum is added to the glass of containers of parenteral solutions to improve resistance to chemical attack and thermal shock (27).

Table 1 shows the reported concentrations of aluminum in calcium gluconate used to prepare parenteral nutrition solutions. Most of these values indicate that the aluminum concentration in this product, when packaged in glass, has averaged ~ 4000 µg/L, and has not decreased over the past three decades. Excluding three high values there has

been a non-significant increase in the aluminum concentration in calcium gluconate packaged in glass over the past three decades (Figure 1).

The average daily administration of aluminum to premature and term infants has been found to be 15 to 25 µg/kg (5, 39, 43, 44), or three to five times the upper boundary of the US FDA guideline, and even higher in Argentina (21). In the United States, until mid-2015, Calcium Gluconate Injection USP had only been available in glass packaging. Pharmacists had few opportunities to minimize aluminum administration in parenteral nutrition solutions, limited to using the most recently obtained product, based on the assumption that the aluminum concentration increases over storage time in the glass container (4, 45), as shown by one study cited in Table 1 (40).

A few reports have shown much less aluminum in calcium gluconate packaged in plastic containers (Table 1) (37, 40). There have been encouragements to abandon glass packaged calcium gluconate in favor of plastic packaging (20, 40). A concern about plastic packaging is the possibility that the product contains leached plasticizers, particularly phthalates such as DEHP (bis(2-ethylhexyl) phthalate, aka: di-2-ethylhexyl phthalate, diethylhexyl phthalate). Phthalates have the potential to cause endocrine (male reproductive system) disruption and neurotoxicity. Infants are the most susceptible population (46, 47). Plasticizers might present a hazard not inherent to glass containers, although the rubber stopper of glass vials could be a source.

The present study was conducted to quantify the aluminum concentration over the shelf life of representative calcium gluconate solutions used to prepare parenteral nutrition solutions, and to determine the initial aluminum concentration and the rate and extent of aluminum concentration increase over storage time. The concentration of phthalates, including DEHP, was determined to ascertain if calcium gluconate provided a risk from exposure to these plasticizers.

Methods:

Multiple containers of calcium gluconate solutions used in the preparation of parenteral nutrition solutions were obtained from three sources in July, 2013; APP Pharmaceuticals, LLC, US, packaged in glass; C.D.M. Lavoisier, France, packaged in glass; and B. Braun, Germany, packaged in plastic. A single vial packaged in glass was obtained from Fresenius Kabi, LLC, US, in May, 2015. Approximately half of the 10 mL calcium gluconate contents of individual containers from APP Pharmaceuticals and B. Braun was clean poured into duplicate seven mL perfluoroalkoxy alkanes (PFA) vials at ~ 1.5 month intervals from July 19, 2013 through August 25, 2015. The interval between sample transfers of the Lavoisier product was ~ every 3 months after the first three samples. The Fresenius Kabi product was similarly transferred on September 8, 2015. The duplicate calcium gluconate samples were analyzed by inductively coupled plasma - mass spectrometry (ICP-MS) to quantify their aluminum concentration. The aluminum content of one of the glass vials from APP Pharmaceuticals and one of the glass ampoules from Lavoisier was determined by ICP-MS. Selected calcium gluconate samples were analyzed by gas chromatography – mass spectrometry (GC-MS) to quantify 8 phthalates. Leachable phthalates from one of the B. Braun plastic containers and the Fresenius Kabi plastic container were determined by GC-MS. Details are reported in the Supplemental Information.

Data analysis:

Outliers of the ICP-MS analysis of aluminum in the calcium gluconate solutions were identified using the Grubb outlier test (http://graphpad.com/quickcalcs/Grubbs1.cfm)

subjecting all results for samples from the same source to the test. One sample of the Lavoisier, four samples of the B. Braun, and two water blanks were identified as outliers and were removed from further data analysis. The average aluminum concentration of the two-hundred fold diluted water blanks (0.45 µg/L) (S.D. = 0.14 µg/L) was subtracted from each two-hundred fold diluted calcium gluconate value. The average aluminum concentration of the duplicate (collected same day) samples was plotted against time, starting with time = 0 when the first samples were transferred from their original containers to the PFA vials (July 19, 2013). Linear regression of aluminum concentration versus time was determined using GraphPad Prism 6 (GraphPad Software, Inc., La Jolla, CA). The aluminum content of the glass containers was compared by a t-test.

Results:

Aluminum analysis:

The instrument detection limit was 0.068 µg/L aluminum. The method detection limit (accounting for required dilution) was 13.6 µg/L aluminum. Aluminum concentration in the two analytical duplicates was 111 and 116% of the original sample. Spike recovery was 104 and 106% for two samples. The aluminum concentration in all samples was greater than the instrument detection limit; the lowest was 0.29 µg/L in a water blank. Aluminum concentration in the calcium gluconate samples is shown in Figure 2. The slope of aluminum concentration versus time for the APP product was significantly different from zero ($F(1,15)$ 58.35, $P < 0.0001$), for the Lavoisier product was not significantly different from zero ($F(1,8)$ 3.75, $P = 0.089$), and for the B. Braun product was ($F(1,14)$ 15.02, $P < 0.0017$). The aluminum concentration in the duplicate samples from the sole Fresenius Kabi sample collected September 8, 2015 averaged 318 µg/L. The aluminum concentrations in the glass vial from APP Pharmaceuticals and the glass ampoule from Lavoisier were not statistically different.

Phthalate analysis:

The blank samples had < 0.02 mg/L of the phthalates. DEHP recovery from spiked water and Calcium Gluconate Injection USP samples was 86 and 91%, respectively. Recovery of the other six phthalates ranged from 76 to 100% (mean = 89%). Recovery of the surrogates ranged from 69 to 98% (mean = 89%). Recovery of p-terphenyl-d14, that eluted closest to most of the phthalates (20.92 minutes), was 89% from water, 93% from the Calcium Gluconate Injection USP blank, and ranged from 74 to 102% (mean =

93%) from the eight tested samples. Recovery of the other two surrogates ranged from 69 to 98% from the blanks and 33 to 98% (mean = 80%) from the eight tested samples. The concentrations of the seven phthalates in the eight tested one mL dichloromethane extracts of four to five mL calcium gluconate were less than the lowest standard (0.1 mg/L), therefore there was < 0.025 mg/L phthalates in all samples. No phthalates were detected in dichloromethane extracts of either the B. Braun or Fresenius Kabi container.

Discussion:

The initial aluminum concentration in calcium gluconate in the APP vials and Lavoisier ampoules was ~ 4800 µg/L (Figure 2), slightly higher than the average reported aluminum concentration in calcium gluconate packaged in glass over the past three decades (Figure 1). A typical initial parenteral nutrition calcium dose for neonates is 2 mEq/kg (48), which would be delivered in 4.3 mL of 10% calcium gluconate (0.465 mEq/mL Ca). 4.3 mL of 4800 µg/L aluminum would deliver ~20 µg/kg/day aluminum, exceeding the FDA guideline of four to five µg/kg/day, and consistent with the average daily administration of aluminum to premature and term infants of 15 to 25 µg/kg, cited in the Introduction. The FDA's labeling requirement that requires a statement of the maximum level of aluminum present at expiry on the container label of small volume parenteral drug products does not resolve the problem of aluminum contamination of parenteral nutrition solutions. It permits aluminum concentrations in small volume parenterals that result in aluminum concentrations in parenteral nutrition solutions in excess of aluminum accumulation associated with central nervous system and bone toxicity. The use of calcium gluconate solutions packaged in glass shortly after their receipt by the end user would not successfully address the aluminum contamination problem.

Aluminum concentration increased over time, as previously reported (40). There is a significant slope (increase of aluminum concentration over time) for the APP product packaged in glass, but not the Lavoisier product also packaged in glass. The increase of aluminum in the Lavoisier product over time (~ 30%) was less than in the APP

product (~ 50%). The Lavoisier product contained ~ 7% calcium gluconate and 3% calcium glucoheptonate (aka gluceptate) compared to 10% calcium gluconate in the APP product. The binding affinity of gluconate for aluminum (49) is large enough to attribute the increase of aluminum over time in calcium gluconate to the leaching of aluminum from the glass containers. No aluminum glucoheptonate stability constant was found in the literature. As the structures of gluconate and glucoheptonate are very similar there is no reason to expect a significant difference in their aluminum binding affinities. The presence of calcium glucoheptonate in the Lavoisier product is unlikely to account for the difference in rate of aluminum increase in these products. The amount of aluminum in the glass vial from APP was not different from the glass ampoule from Lavoisier (Supplemental Information). Package inserts do not inform about the type of glass. The USP monograph for Calcium Gluconate Injection states that the solution should be preserved in single-dose containers, preferably of Type I glass. Type I glass is used to manufacture ampoules. It is not clear if there are differences in the glass composition of the APP and Lavoisier product containers that influenced the rate of aluminum increase over time in calcium gluconate stored in those containers. Irrespective of the glass source, using a calcium gluconate solution from a glass package that has been on the shelf for some time exacerbates the problem of aluminum contamination.

Switching calcium gluconate from glass to plastic packaging does not remove all parenteral nutrition component sources of aluminum. Plastic packaged calcium gluconate contains measurable aluminum (Table 1 and results of this study). Other

components of parenteral nutrition solutions and intravenous therapy of neonates are also contaminated with aluminum. Three of concern are phosphates, heparin, and albumin. Table 2 contains the aluminum concentrations in phosphates, heparin, and albumin reported in the publications cited in Table 1. There are reports of aluminum concentrations in 18 phosphate solutions in which the phosphate concentration is sufficiently reported to calculate aluminum (in µg) in one mmol of phosphate (2, 17, 22, 25, 28, 30, 33, 34, 41), the phosphate dose to initiate parenteral nutrition per kg patient body weight (48). The median aluminum concentration of the 18 solutions is 1400 µg/L, which would deliver 1.4 µg aluminum per mmol phosphate. Compared to the ~ 20 µg of aluminum from calcium gluconate that would be in parenteral nutrition solution for a 1 kg neonate (above), calcium gluconate is a greater contributor. The aluminum contribution from heparin to parenteral nutrition solutions is negligible, given its suggested concentration in parenteral nutrition solutions is 1 U/mL (48) . As albumin is not a routine component of parenteral nutrition solutions its contribution compared to calcium gluconate is not made. However, large, repeated albumin doses might present a significant amount of aluminum.

The problem of aluminum contamination of calcium gluconate can be largely avoided by the use of plastic packaging, as has been suggested (20, 40). The present results support that suggestion.

A concern for products packaged in plastic is the potential release of plasticizers from the container into the product. The phthalates are of particular concern, especially for infants. The maximum allowable dose for DEHP by intravenous injection to neonatal

infant boys (birth to 28 days of age) is 210 µg/day (50). Based on the typical initial parenteral calcium dose for a neonate of average weight (3.4 kg), which would be delivered in ~ 14.6 mL of 10% calcium gluconate, the maximum allowable level of DEHP in calcium gluconate is ~ 15 µg/mL. None of the samples had > 0.2% of this level.

The results of the present study, and prior reports of aluminum contamination of calcium gluconate packaged in glass and plastic, and the lack of phthalate plasticizers in calcium gluconate packaged in plastic, support the suggestions that glass-packaged calcium gluconate should be avoided as a component of parenteral nutrition solutions delivered to patients with impaired kidney function. The recent introduction in the US of plastic container packaged calcium gluconate should enable the preparation of parenteral nutrition solutions with considerably reduced aluminum content, to the benefit of patients with impaired kidney function, including premature neonates.

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Figure legends

Figure 1. Aluminum concentration of calcium gluconate packaged in glass. The values are from Table 1 with the exclusion of 10,695 reported by (35) and values reported by (18). Line is best fit of the data points.

Figure 2. Aluminum concentration in 10% calcium gluconate from single lots collected over time. Values are the mean of the duplicate samples collected on and after the date of the first sample (time = 0). Note the different Y axis scale for the B. Braun product. Line is best fit of the results.

Figure 1

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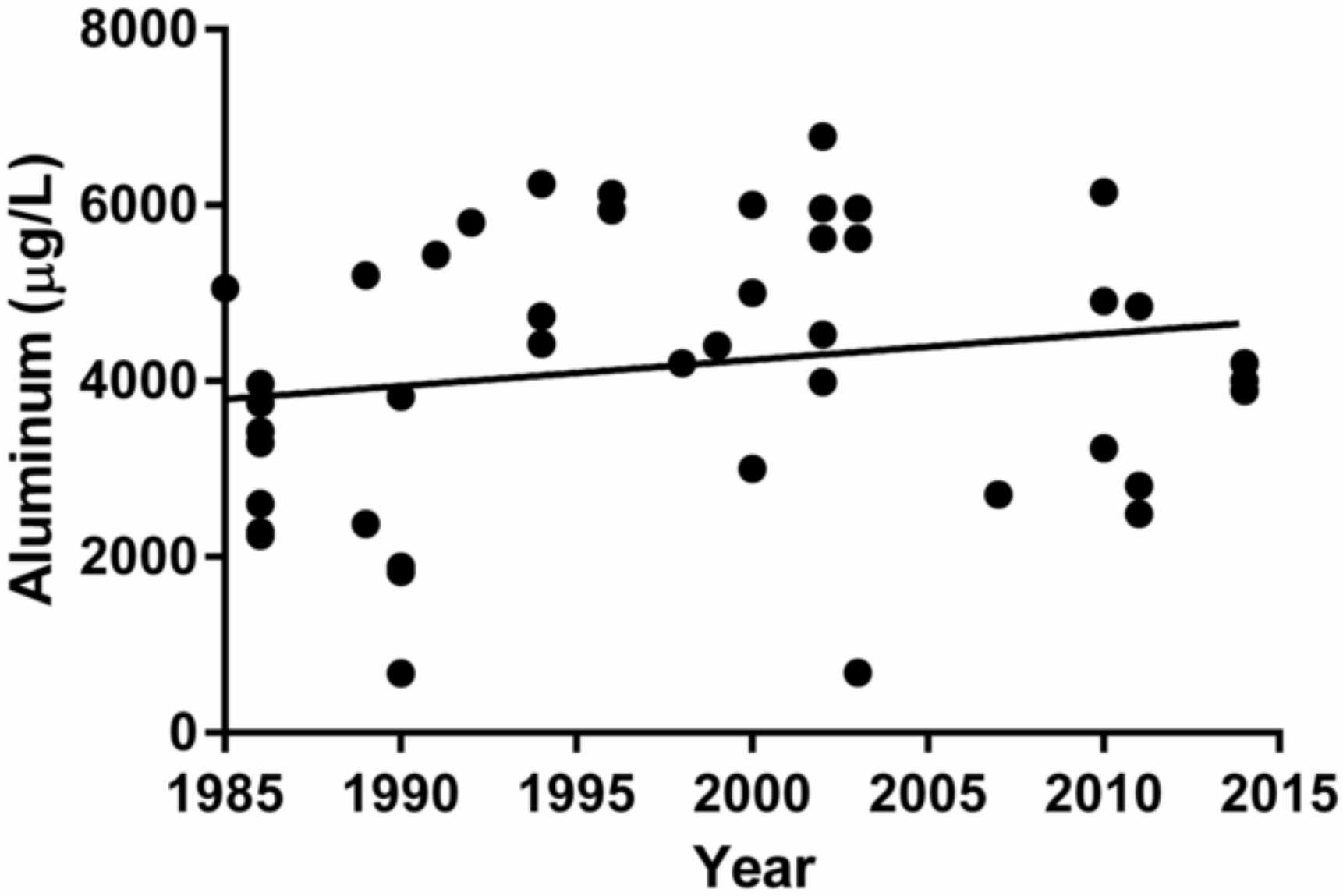

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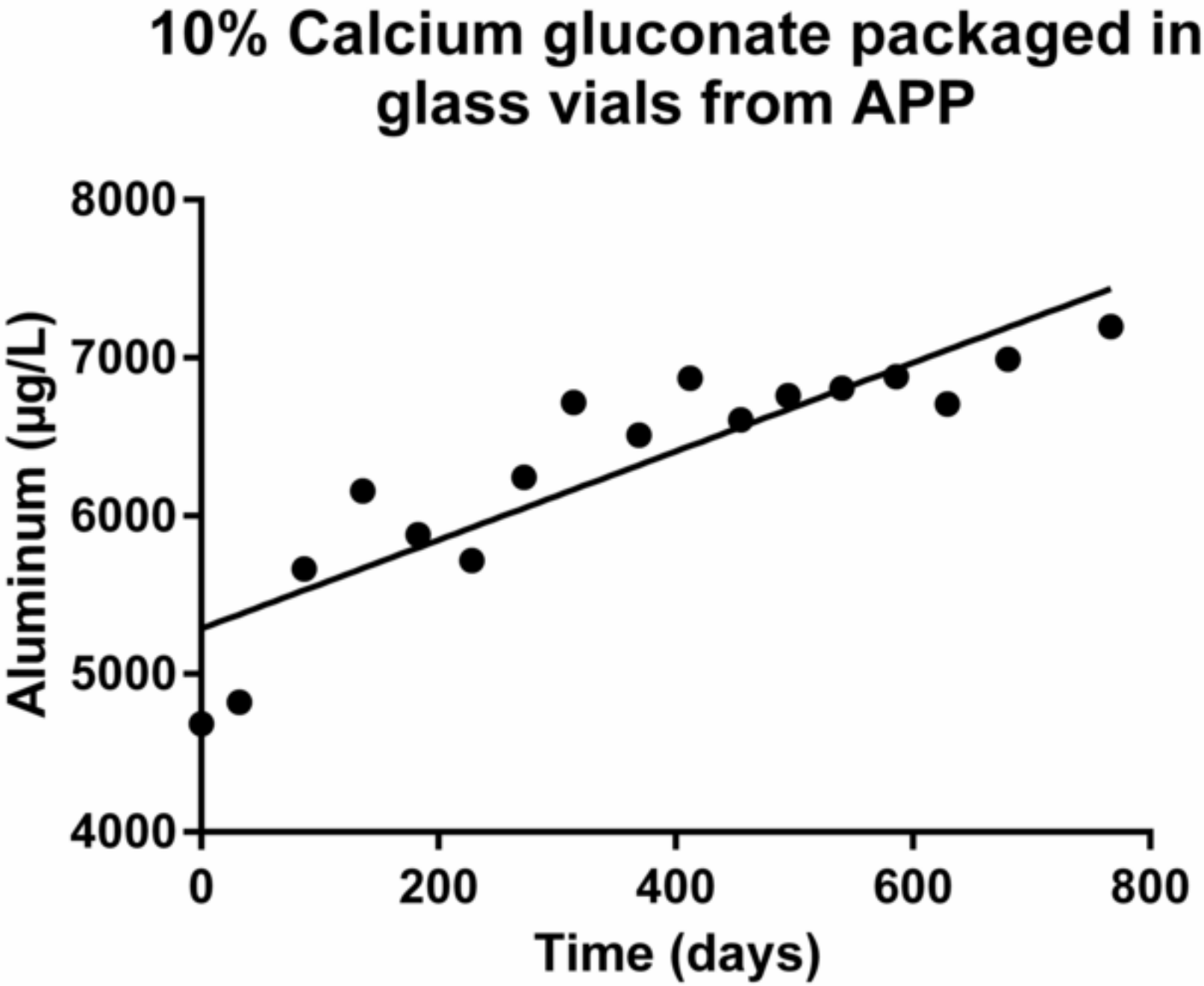

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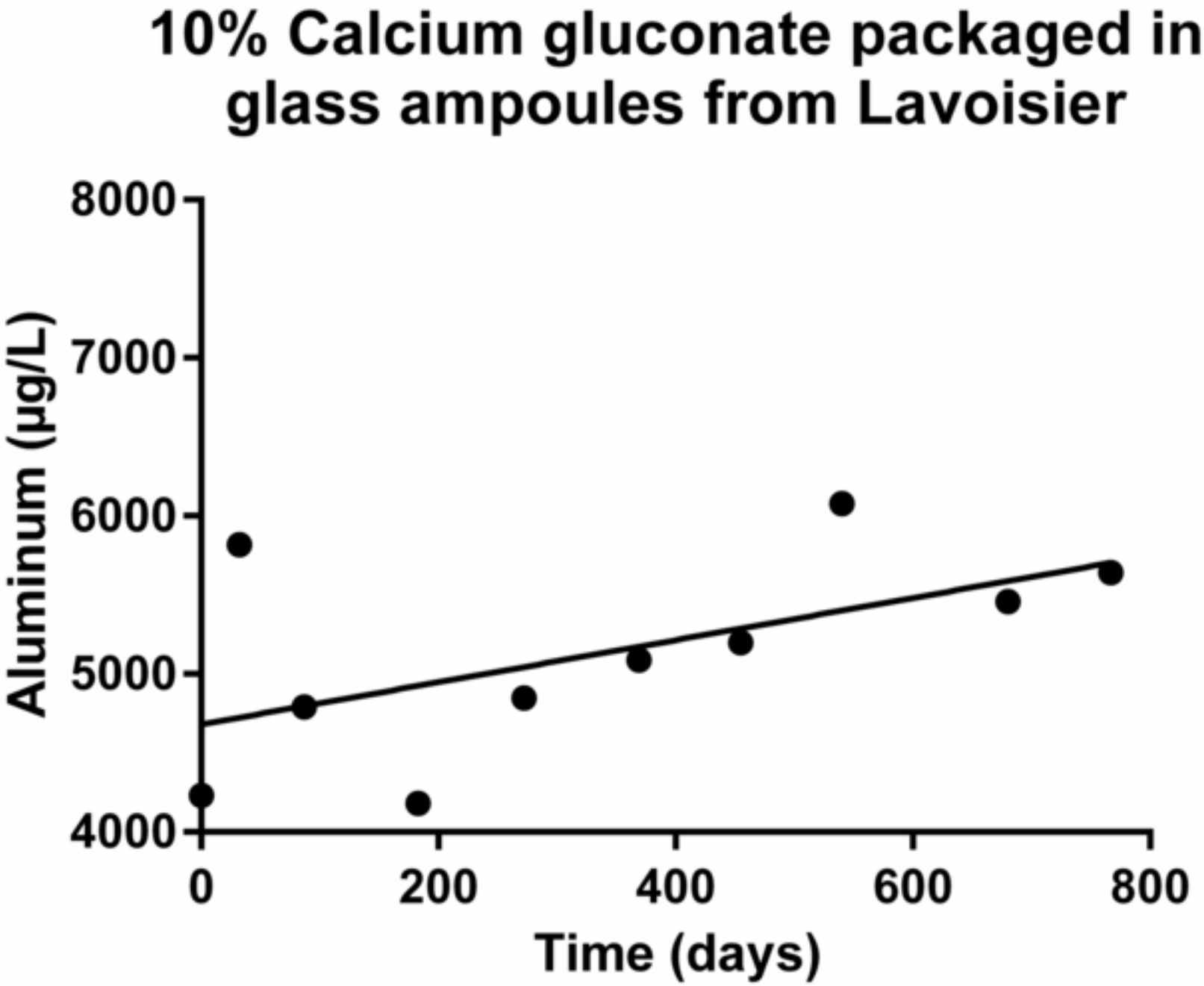

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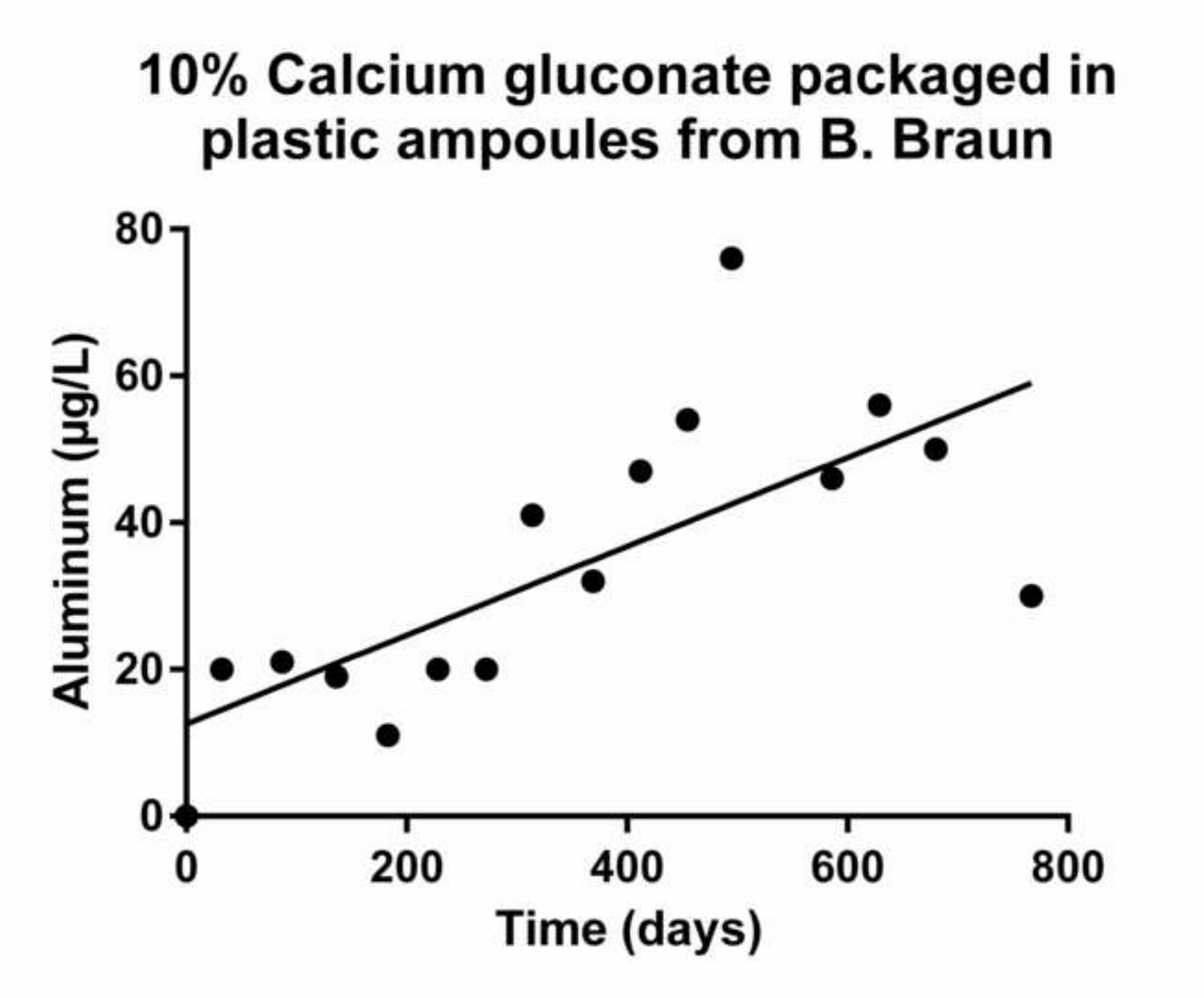

Table 1. Reported aluminum concentrations in calcium gluconate.

Source (as stated in the reference)	Aluminum concentration (µg/L)	Reference
Packaged in glass		
Calcium gluconate 10%	5056	(2)
Calcium, Sandoz	3753	(28)
Calcium gluconate, Lymphomed	2245 (range 2000 to 2586)	(13)
Calcium gluconate, Invenex	2592 & 2610	
Calcium gluconate, Elkins Sinn	3973 (range 1095 to 5565)	
Calcium gluconate, IMS	3299	
Calcium gluconate, American quinine	2286	
10% Calcium gluconate, Phoenix	3430	(29)
Calcium gluconate	4850	(30)
Ca gluconate, Lymphomed	2375 (range 2000 to 2750)	(31)
Calcium gluconate	1100 to 5600	(14)
Calcium gluconate 10%, Pharmacie Centrale AP Paris	675 & 1830	(32)
Calcium gluconate 10%, Arguettant	1890	
Calcium gluconicum, Braun	3825	(3)
Calcium gluconate, Palex	5437	(33)
Calcium gluconate 0.22 mmol/mL, David Bull Labs. Pty. Ltd.	5800	(34)
Calcium 10%, Sandoz	4900	
Calcium gluceptate 10%	4800	

Table 1. Reported values of the aluminum concentration in calcium gluconate. (cont.)

Calcium 10%, Braun	4734 & 6243	(35)
Calcium 20%, Braun	10,695	
Calcium-gluconat 10%, Pharma Hameln	4421	
Calcium gluconate 10%, Meram	5940	(15)
Calcium gluconate 10%, Aguettant	5940	
Calcium gluconate, AP Paris	6130	
Calcium gluconate, American Regent	4201 ± 267	(16)
10% Calcium gluconate, Commercial Polfa	4400	(36)
Calcium gluconate 10% (10 ml vial)	5000	(37)
Calcium gluconate 10% (100 ml vial)	3000	
Calcium gluconate 20% (10 ml vial)	6000	
Halex Istar (lot 1)	5621	(22)
Halex Istar (lot 2)	6781	
Ariston	5960	
B. Braun	4530	
Elkins Sinn	3987	
Calcium gluconate 10%	5621 & 5960	(25)
Calcium gluconate 5%, PCH, Paris, France	680	(17)
Calcium gluconate 10%	2712 (range 2240 to 3040)	(38)
Calcium gluconate 10%, Hypofarma	9205 & 19,400	(18)
Calcium gluconate	3234	(39)
Calcium gluconate 10% (new)	4910	(40)
Calcium gluconate 10% (old)	6150	

Table 1. Reported values of the aluminum concentration in calcium gluconate. (cont.)

Calcium gluconate 100 mg/ml, American Pharmaceutical Partners	2812 (range 1969 to 3495)	(41)
Calcium gluconate 100 mg/ml, American Regent	2487 (range 1928 to 2887)	
Calcium gluconate 10%	range: 4000 to 4400	(42)
Calcium gluconate 10%, PISA Farmaceutica	3890	(20)
Calcium gluconate 20%	4000 (range 3100 to 4200)	(21)
Packaged in plastic		
Calcium gluconate 10%, Braun	105	(37)
Calcium gluconate 20%, Braun	195	
Calcium gluconate 10%	27 & 30 (new) 31 & 33 (old)	(40)

Table 2. Reported aluminum concentrations (µg/L) in phosphates, heparin and albumin.

Phosphate		Heparin	Albumin	Reference
Potassium phosphate 3 M	16,598	1000 U/mL 684 5000 U/mL 359 10,000 U/mL 468	25% 1822	(2)
Sodium phosphate 3 M	5056			
Potassium phosphate 1 M	486		1296	(28)
Potassium phosphate	92 & 2189 (2069 to 2301)		25% 914 & 2364 (1116 to 5849)	(13)
Sodium phosphate	6 & 2236 (2026 to 2370)			
Potassium acid phosphate	1882			(29)
Potassium acid phosphate 13.6% (1 M)	2154			(30)
Potassium phosphate	2754 (2025 to 7128)			(31)
Sodium phosphate	65 (ND* to 284)			
Potassium phosphate	90 to 2300			(14)
Sodium phosphate	< 5 to 2370			
			5% 164 20% 28	(3)
Potassium phosphate	9539			(33)
Monopotassium phosphate 1 M	1533			
Dipotassium phosphate 1 M	3615			
Monosodium phosphate 1 M	1451			

Table 2. Reported aluminum concentrations (µg/L) in phosphates, heparin and albumin. (cont.)

Potassium acid phosphate 1 M	2387	100 to 25,000 U/mL 6 to 31		(34)
Potassium dihydrogen phosphate 0.54 g and di- potassium hydrogen phosphate 1.83 g in 10 mL	7689			
Potassium phosphate, Braun	18 & 188	Sodium heparin 25,000 IE/5 mL 70	5% 90 ± 61 20% 57 ± 45	(35)
Potassium phosphate pfrimmer, Kabi	2826 & 3421			
Sodium phosphates, American Regent	6288			(16)
Potassium phosphates, not commercial	9800			(36)
Sodium potassium phosphates, Addiphos Pharmacia	13,000 to 41,600			
Potassium phosphate, Aster, B. Braun, & Fresenius, 2 mEq/mL	689 ± 348	5000 IU/mL 481 ± 275 5000 IU/0.25 mL 165 ± 27	20% 94 & 235	(22)
Sodium phosphate, Abbott & Geyer, 0.5 M	568 ± 320			
Potassium phosphate, 2 mEq/mL	988 & 1325	5000 IU/mL 732 & 738	20% 149 & 644	(25)
Sodium phosphate, 0.5 mol/L	879 & 933			
Dipotassium phosphate 1.7 M	240			(17)
		Sodium heparin 1% 108 (range 106 to 112)	20% 221 (range: 195 to 231)	(38)

Table 2. Reported aluminum concentrations (µg/L) in phosphates, heparin and albumin. (cont.)

Potassium phosphate, American Pharmaceutical Partners	8250			(39)
Sodium phosphate, American Regent	622			
Potassium phosphate 3 M	4040 & 9972			(41)
Sodium phosphate 3 M	29 & 3242			
Potassium phosphate, PISA Farmaceutica	3780			(20)
Sodium phosphate, PISA Farmaceutica	1780			
Sodium phosphate	4460 (range 4638 to 4809)			(21)

* = not detected

Supplemental Information to: Aluminum and phthalates in calcium gluconate; contribution from glass and plastic packaging

Materials and Methods:

Materials:

Twenty-five 10 ml glass vials of Calcium Gluconate Injection, USP (APP Pharmaceuticals, LLC, Schaumburg, IL; Product Code 31110, NDC No. 63323-311-10, Lot 6005911, Expiration April, 2015), ten 10 ml glass ampoules of Gluconate de Calcium Lavoisier 10% (C.D.M. Lavoisier, 75017 Paris, France; Lot 2A154, Expiration September, 2015), and twenty 10 ml plastic ampulles of Calciumgluconat 10% B. Braun Injektionslösung (B. Braun Melsungen AG – 34209 Melsungen, Deutschland; Lot 12417011; Expiration September, 2015) were obtained in July, 2013. One 10 plastic vial of Calcium Gluconate Injection, USP (Fresenius Kabi, LLC, Lake Zurich, IL; Product Code 31110, NDC No. 63323-311-19, Lot 6009399, Expiration March, 2016), was obtained in May, 2015. EPA Method 506 Phthalates Mixture containing bis(2-ethylhexyl) adipate, bis(2-ethylhexyl) phthalate (DEHP), butyl benzyl phthalate, di-n-butyl phthalate, dimethyl phthalate, diethyl phthalate, and di-n-octyl phthalate (Ultra Scientific); an internal standard mix containing acenaphtene-d10, chrysene-d12, 1,4-dichlorobenzene-d4, naphthalene-d8, perylene-d12, and phenanthrene-d10 (Restek); and an EPA Method 3500 (SW-846) and CLP–Semi-Volatiles Base/Neutrals Surrogates mixture containing 2-fluorobiphenyl, nitrobenzene-d5, and p-terphenyl-d14 (Restek) were obtained from VWR.

Methods:

Sample collection:

Approximately half of the 10 ml calcium gluconate contents of individual containers from APP Pharmaceuticals and B. Braun was clean poured into duplicate seven ml perfluoroalkoxy alkanes (PFA) vials (Savillex, Eden Prairie, MN) at ~ 1.5 month intervals. Approximately half of the 10 ml contents of ampoules from C.D.M. Lavoisier was clean poured into duplicate seven ml PFA vials at ~ 1.5 month intervals initially, then ~ at three month intervals. Approximately half of the 10 ml contents of the Fresenius Kabi product was clean poured into duplicate seven ml PFA vials September 8, 2015. Sample transfer was conducted in a Class 100 laminar flow hood. The PFA vials had been pre-cleaned by soaking in 10% nitric acid then 5 mm EDTA, and triple rinsed with 18.2 MΩ-cm water. Water (18.2 MΩ-cm) from a Barnstead NANOpure water polishing system, stored in a 500 ml FEP bottle that had been triple cleaned with trace metal grade nitric acid, was clean-poured into duplicate PFA vials on many of the same occasions calcium gluconate samples were transferred, to serve as a blank. All samples before and after transfer to PFA vials were stored at room temperature throughout the study.

Aluminum quantification in calcium gluconate solutions by ICP-MS:

Immediately prior to analysis, samples were heated to solubilize precipitation and then diluted two-hundred-fold with 1% (v/v) concentrated ultra-pure nitric acid. Results were compared to a standard curve created from an Inorganic Ventures (Christiansburg, VA, USA) multi-element ICP-MS standard. To validate the calibration curve, a standard of

known concentration was prepared from a multi-element standard of a different lot number and analyzed as an unknown. Two samples were diluted and analyzed a second time as sample dilution replicates. Spike recovery was determined by adding 10 µL of the 10 mg/L calibration standard to two 10 mL samples, adding 10 µg/L aluminum. Scandium was added as the internal standard to all samples and standards for the aluminum analyses at the same concentration (approximately five µg/L). The instrument detection limit was calculated as three times the standard deviation of the eleven reagent blanks. Care was taken to minimize metal contamination, including use of metal-free centrifuge tubes, trace metal grade nitric acid, and 18.2 MΩ-cm water. All dilution procedures were conducted in a class 100 laminar flow hood. Samples were analyzed by ICP-MS (Agilent 7500cx, Santa Clara, CA, US).

Aluminum analysis of glass vials by laser ablation ICP-MS:

A glass vial that contained Calcium Gluconate Injection, USP from APP Pharmaceuticals and a glass ampoule that contained Gluconate de Calcium from Lavoisier were wrapped in paper and shattered using a hammer. Samples from the bottom of each vial were collected and rinsed thoroughly with deionized water. The samples were them mounted on microscope slides using double sided tape with the inside surface of the bottom of the vials facing up. The samples were analyzed using a 213 nm laser ablation system (CETAC Technologies LSX-213, Omaha, NE, USA) coupled to an inductively coupled plasma mass spectrometer (Agilent Technologies 7500CX, Santa Clara, CA, USA). Duplicate samples from each vial were ablated with 100 laser shots at a frequency of 20 Hz within a 200 µm spot. Standardization was

performed by ablating standards with known aluminum concentrations in a calcium carbonate matrix in the same manner. These standards were prepared by mixing a known mass of aluminum contained within an ICP-MS standard solution (Inorganic Ventures, Christiansburg, VA, USA) with a known mass of calcium carbonate, drying the mixture, and pressing it into pellets with a pellet press. We were not able to make or obtain standards with a glass matrix that had known aluminum concentrations. Consequently, although the numbers are precise, they may not be accurate because laser ablation is highly matrix dependent. The true aluminum concentration may be different by as much as 10-fold. The aluminum concentration in the glass of the glass vial from APP was 2082 ± 170 (SD) mg/kg (~ 0.2%) and in the glass of the glass ampoule from Lavoisier: 2224 ± 228 (SD) mg/kg (~ 0.2%). These results indicate there was no significant difference in the aluminum concentration between these two containers, but must not be cited as accurate determinations of aluminum concentration. Reports of the aluminum concentration in glass vials include 5.8% Al_2O_3 (or 3.1% Al) in Type I USP borosilicate hard glass and 1.9% Al_2O_3 (or 1% Al) in Type II USP soft glass (1), 0.61 to 3.01% aluminum (2), and 5% Al_2O_3 (or 2.6% Al) in Type I USP glass (3).

Phthalate analysis in calcium gluconate solutions by gas chromatography – mass spectrometry:

A method was developed based on a modification of EPA method 8270D that separated the seven components of the phthalates mixture, with retention times from 12.95 to 25.12 minutes. DEHP eluted at 23.65 minutes. The method also separated the

phthalates from the six internal standard components, which had retention times from 6.74 to 26.49 minutes, and the three surrogates, which had retention times from 7.85 to 20.92 minutes. A calibration curve was prepared containing 0.1, 1.0, 2.5, 5.0, 7.5, and 10 ppm phthalates. Five blanks were prepared (water, water exposed to pipette tip from the lot used to prepare the dilutions of calcium gluconate for ICP-MS aluminum quantification [above], Calcium Gluconate Injection USP, Calcium Gluconate Injection USP in PFA vial, and Calcium Gluconate Injection USP exposed to pipette tip). Phthalate recovery was determined from water and Calcium Gluconate Injection USP in PFA vials, by addition of 5 ppm phthalates. Samples of 10% calcium gluconate from APP Pharmaceuticals, Lavoisier, and B. Braun from the last collection date (August 25, 2015) and the Fresenius Kabi sample were analyzed for their phthalate content. Five µL of 1000 ppm surrogate mix was added to the blanks, spiked samples, and 10% calcium gluconate samples (four to five ml) which were then extracted by adding three ml dichloromethane, shaken vigorously for five minutes, then transferring the organic layer to a separate vial. The extraction step was repeated and the extracts were combined and evaporated under a gentle stream of nitrogen to near dryness, the vial was rinsed and brought to a final volume of one ml with dichloromethane. Two µL of 2000 ppm internal standard mix was added to each of the extracts and calibration curve components. The samples were analyzed on a Varian CP-3800 GC with a Varian Saturn 2200 MS.

Phthalate analysis in leachates from plastic containers by gas chromatography – mass spectrometry:

The B. Braun plastic container that provided the last measured sample, on August 25, 2015, and the Fresenius Kabi plastic container were used to determine if phthalates would leach from the plastic. A portion of the bottom of each container was removed and weighed (B. Braun 0.311 g; Fresenius Kabi 0.371 g) and placed in 24 ml vials with five ml of dichloromethane. The vials were agitated by sonication and vortex mixing for 10 minutes. One ml of the extract was transferred to an autosampler vial adding two µL of internal standard mix. Samples were analyzed by GC-MS as described above for the detection of phthalates.

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