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Development and validation of an ion chromatography method for the determination of phosphate-binding of lanthanum carbonate☆

Raghu Samy[a], Patrick J. Faustino[a], Wallace Adams[b], Lawrence Yu[b], Mansoor A. Khan[a], Yongsheng Yang[a,*]

[a] Division of Product Quality Research, Office of Pharmaceutical Science, Food and Drug Administration, Life Science Building 64, 10903 New Hampshire Avenue, Silver Spring, MD 20993, USA
[b] Office of Generic Drugs, Food and Drug Administration, 7519 Standish Place, Rockville, MD 20855, USA

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ABSTRACT

Lanthanum carbonate is indicated to reduce serum phosphate in patients with end stage renal disease (ESRD). When given orally, lanthanum carbonate dissociates in the acid environment of the upper gastrointestinal tract to release lanthanum ions. The free lanthanum ions bind with dietary phosphate released from food during digestion to form highly insoluble lanthanum–phosphate complexes which prevent the absorption of phosphate, consequently reduce the serum phosphate. In order to evaluate the *in vitro* binding capacity of lanthanum carbonate, a simple and efficient ion chromatography (IC) method was developed and validated for determination of phosphate across the pH range encountered in the gastrointestinal tract. Chromatographic separation was achieved on a Dionex ICS-2000 IC system using a Dionex AS16, IonPac (4 mm × 250 mm) analytical column and Dionex AG16, IonPac (4 mm × 50 mm) guard column. Column temperature was maintained at 30 °C. Injection volume was 10 μL. The compounds were eluted isocratically at a flow rate of 1 mL/min and detected by suppressed conductivity. The analytical method was validated according to USP Category I requirements. The validation characteristics included accuracy, precision, quantification limit, linearity, and stability. The intra-day accuracy ranged from 89% to 103% for the solutions of pH 1.2–6.8. The intra-day precision (RSD) ranged from 0.6% to 3.7% for the solutions of pH 1.2–6.8. The analytical range was linear from 2 to 200 ppm (mg/L). The R^2 ranged from 0.9998 to 1.0. This method was found to be simple, robust, sensitive, specific, and accurate. It has been successfully applied for determination of phosphate binding to lanthanum carbonate over the human gastrointestinal pH range at different time-points (from 0.5 to 24 h).

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1. Introduction

Hyperphosphatemia is an electrolyte disturbance in which there is an abnormally elevated level of phosphate in serum. It develops in the majority of patients with end stage renal disease (ESRD) and is associated with secondary hyperparathyroidism, metabolic bone disease, soft tissue calcification and possibly cardiovascular calcification [1–3]. Adequate control of serum phosphate remains a cornerstone in the clinical management of patients with ESRD. These measures include dietary phosphorus restriction, dialysis and oral phosphate binders [4]. Given the limitations of restricting phosphate in the diet and the inadequate removal by conventional dialysis regimes, the use of phosphate binders is therefore a major therapeutic consideration to maintain phosphate balance and to prevent hyperphosphatemia [5–6]. Because of toxicity, aluminum-containing phosphate binders are no longer used [7–8]. Calcium based phosphate binders, such as calcium carbonate and calcium acetate, have no problems associated with aluminum toxicity, but they often result in hypercalcemia that may be linked to cardiovascular calcification [9–11]. Recently, the aluminum-free and calcium-free phosphate binding agents including sevelamer hydrochloride and lanthanum carbonate have been approved for the prevention and treatment of hyperphosphatemia in patients with ESRD [12–16].

When given orally lanthanum carbonate dissociates in the acid environment of the upper gastrointestinal tract to release lanthanum ions which are trivalent cations with a high affinity for oxygen-donor atoms, especially phosphates. These cations bind to ingested phosphates to form insoluble, non-absorbable lanthanum–phosphate complexes, which are excreted in the feces [17–18]. The oral bioavailability of lanthanum in humans is less

☆ This scientific contribution is intended to support regulatory policy development. The views presented in this article have not been adopted as regulatory policies by the Food and Drug Administration at this time.

* Corresponding author. Tel.: +1 301 796 0024; fax: +1 301 796 9816.
E-mail address: yongsheng.yang@fda.hhs.gov (Y. Yang).

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Table 1
Parameters of calibration curve ($n \geq 9$).

Solutions	Linear range (ppm)	Calibrators	R^2 value	Slope	y-Intercept
pH 1.2	2.0–200.0	7	1.0	0.034	−0.021
pH 4.5	2.0–200.0	7	0.9998	0.033	−0.066
pH 6.8	2.0–200.0	7	1.0	0.034	−0.015

than 0.002% [18–19], suggesting that pharmacokinetics have little relevance to the efficacy of lanthanum carbonate. Therefore, an *in vitro* determination of phosphate-binding capacity becomes a useful approach to assess the efficacy of lanthanum carbonate. For this purpose, it is necessary to have a simple, sensitive, specific, and validated method to detect phosphate in binding media with different pH levels encountered in the gastrointestinal tract. The objective of this study was to develop and validate an ion chromatography method to be used to determine phosphate-binding to lanthanum carbonate over the human gastrointestinal pH range at different time-points (from 0.5 to 24 h).

2. Experimental

2.1. Chemicals, reagents and product

Phosphate certified standard (1000 μg/mL in water, 1000 ppm) was purchased from High-Purity Standards (Charleston, SC). Hydrochloric acid, sodium phosphate monobasic, monohydrate and boric acid were purchased from Fisher Scientific (Fair Lawn, NJ). Sodium acetate and acetic acid were purchased from Sigma (St. Louis, MO). Sodium tetraborate decahydrate and lanthanum carbonate hydrate were purchased from Aldrich (Milwaukee, WI). Lanthanum carbonate drug product was purchased from Washington Drug Wholesale (Savage, MD). Deionized water was supplied in house by a Millipore Milli-Q System (Bedford, MA). All other chemicals were of reagent grade.

2.2. Preparation of pH media

The solution of 0.1 N HCl (pH 1.2) was prepared by adding 5.1 mL of concentrated HCl (37.4%) into 594.9 mL of deionized water. The 0.1 M acetate buffer with pH 4.5 was prepared from acetic acid and sodium acetate. The 0.1 M borate buffer with pH 6.8 was prepared from boric acid and sodium tetraborate decahydrate.

2.3. Preparation of calibration standards and quality control standards

Phosphate certified standard (1000 μg/mL in water, 1000 ppm) was used as standard stock solution. Note: 1 mM phosphate = 95 ppm of phosphate. Seven calibration standard solutions were prepared by transferring 0.02, 0.1, 0.2, 0.3, 0.5, 1.0 and 2.0 mL of stock solution to 10-mL volumetric flasks and bringing to the volume with the corresponding pH solution to obtain the final concentrations of 2, 10, 20, 30, 50, 100 and 200 ppm phosphate, respectively. Three standard curves including standard blanks were prepared fresh daily.

Table 2
Quality control: accuracy (%, $n \geq 15$).

Quality control standards	Phosphate concentration (ppm)			
	2.0	10.0	50.0	200.0
pH 1.2	103.3	99.0	98.6	99.6
pH 4.5	88.8	90.6	94.4	100.4
pH 6.8	99.0	98.5	99.1	99.7

Table 3
Quality control: precision (%RSD, $n \geq 15$).

Quality control standards	Phosphate concentration (ppm)			
	2.0	10.0	50.0	200.0
pH 1.2	2.3	1.2	1.2	0.6
pH 4.5	3.7	2.4	1.5	0.7
pH 6.8	3.6	1.1	0.8	0.6

The quality control (QC) phosphate stock solution (1000 μg/mL in water, 1000 ppm) was prepared by dissolving 1.453 g of sodium phosphate monobasic, monohydrate in 1000 mL of deionized water. The lower limit of quantification (LLOQ) of 2 ppm, low QC of 10 ppm, intermediate QC of 50 ppm and high QC of 200 ppm were prepared by transferring 0.02, 0.1, 0.5 and 2.0 mL of stock solution to 10-mL volumetric flasks and then adding the corresponding pH solution to the volume. Five standards at each QC level were prepared freshly.

2.4. Preparation of samples

Eight concentrations of phosphate 100, 200, 300, 400, 500, 600, 800 and 1000 mM were prepared in water. 0.1 g of lanthanum carbonate drug substance or the drug product equivalent to 0.1 g of lanthanum carbonate was accurately weighed and transferred to a 100-mL glass flask. Then 45 mL of pH solution and 5 mL of phosphate solution were added to the flask. The final concentration of phosphate ranged from 10 to 100 mM. The flask was tightly stopped and incubated in a shaking water bath at 37 °C at 75 shakes/min. The samples were taken at 0.5, 1, 2, 3, 4, 6, 8 and 24 h and filtered through a 0.2 μm Acrodisc® filter (Gelman Laboratory, MI). The clear-filtered solution was diluted by 50- to 100-fold and 1.0 mL aliquot of sample was used for phosphate analysis. All samples were prepared in triplicate.

2.5. Analytical method for phosphate

All standards and samples were analyzed by an IC system which consisted of a Dionex ICS-2000 (Bannockburn, IL) equipped with a dual piston pump, degasser, suppressor, column heater, autosampler, and detector. Data collection and analysis were performed using Chromeleon software, version 6.8 (Bannockburn, IL). Separation was achieved on a Dionex A16, IonPac (4 mm × 250 mm) column with a guard column. The mobile phase was 35 mM KOH delivered isocratically at a flow rate of 1 mL/min. The column temperature was maintained at 30 °C. The injection volume was 10 μL and detection was by suppressed conductivity. Suppression was achieved with anion suppressor (ASRS 300 4 mm) from Dionex. Suppression current was set at 87 mA.

3. Results and discussion

3.1. Analytical method validation

Ion chromatography was first introduced in 1975 as a new analytical method and has become a well-established chromato-

Table 4
System suitability test results ($n \geq 18$).

Parameters	Specification	pH 1.2	pH 4.5	pH 6.8
Capacity factor (k')	>1.0	1.31	1.25	1.28
Area (%RSD)	<2.0%	0.38	0.64	0.80
Retention time (%RSD)	<0.5%	0.03	0.1	0.07
Plates	>3000	5909	5940	5865
USP tailing	<2.0	1.13	1.15	1.13

Table 5
Robustness test results 1: variations of flow rate.

System suitability parameter[a]	Specification	Flow rate (0.9 mL/min)	Flow rate (1.0 mL/min)	Flow rate (1.1 mL/min)
Capacity factor (k')	>1.0	1.35	1.46	1.31
Area (%RSD)	<2.0%	0.41	0.54	0.78
Retention time (%RSD)	<0.5%	0.14	0.10	0.13
Theoretical plates	>3000	9688	9116	9139
USP tailing	<2.0	1.13	1.13	1.14

[a] System suitability standard in diluted HCl (pH 1.2).

Table 6
Robustness test results 2: variations of injection volume.

System suitability parameter[a]	Specification	Injection volume (5 μL)	Injection volume (10 μL)	Injection volume (15 μL)
Capacity factor (k')	>1.0	1.35	1.46	1.35
Area (%RSD)	<2.0%	0.88	0.54	0.35
Retention time (%RSD)	<0.5%	0.06	0.10	0.08
Theoretical plates	>3000	9603	9116	9588
USP tailing	<2.0	1.12	1.13	1.13

[a] System suitability standard in diluted HCl (pH 1.2).

Table 7
Robustness test results 3: variations of KOH concentration.

System suitability parameter[a]	Specification	KOH (34 mM)	KOH (35 mM)	KOH (36 mM)
Capacity factor (k')	>1.0	1.48	1.46	1.25
Area (%RSD)	<2.0%	0.79	0.54	0.68
Retention time (%RSD)	<0.5%	0.02	0.10	0.08
Theoretical plates	>3000	9407	9116	9455
USP tailing	<2.0	1.14	1.13	1.14

[a] System suitability standard in diluted HCl (pH 1.2).

graphic [20]. For the past 30 years it has been widely used for the determination of inorganic anions and small organic anions [21]. However there are few IC methods for phosphate that are utilized for pharmaceutical assessment. Deborba used IC to simultaneously evaluate citrate and phosphate in pharmaceutical dosage formulations [22]. IC was also used by Swearingen and Mazzeo [23–24] as a phosphate analytical method to determine the binding parameter constants of sevelamer hydrochloride; however no published IC method is available for the determination of phosphate binding to lanthanum carbonate under the physiologically relevant pH conditions in the gastrointestinal tract. Our efforts were focused on developing a simple, sensitive, and specific IC method for phosphate. By modifying Swearingen and Mazzeo's method [23–24], we have established and validated an IC method for the determination of phosphate in media with various pH values. Modifications included utilizing different mobile phase, new IC analytical and guard columns, reduced injection volume and an elevated temperature condition that allowed for the development of time efficient method while enhancing the resolution of phosphate from other anions such as chloride and carbonate.

The method was validated according to the USP category I requirements [25]. The following validation characteristics were addressed: the stability of the solutions, linearity, range, accuracy, precision, specificity, and robustness.

3.1.1. Stability of the solutions

The response factor of phosphate standard solutions was found to be unchanged for up to 90 days. Less than 0.1% concentration difference was found between the solutions freshly prepared and those aged 90 days. The solutions can therefore be used within this period without the results being affected.

3.1.2. Linearity and range

Calibration curve was linear over the validated range of 2–200 ppm for the solutions with pH level of 1.2–6.8. The results summarized in Table 1 show an excellent correlation between the peak area and concentration of phosphate over the analytical range on 15 different days performed over a period of 6 months ($R^2 \geq 0.9998$).

3.1.3. Accuracy and precision

The accuracy is the closeness of mean test results obtained by the method to the true value of analyte, calculated as the percentage of recovery. The precision describes the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of a homogenous sample and calculated as relative standard deviation (RSD). The results of intra-day accuracy and precision in the study are compiled in Tables 2 and 3. The mean accuracies ranged from 89% to 103% for three levels of pH

Table 8
Robustness test results 4: variations of column temperature.

System suitability parameter[a]	Specification	Column temperature (25 °C)	Column temperature (30 °C)	Column temperature (35 °C)
Capacity factor (k')	>1.0	1.11	1.46	1.63
Area (%RSD)	<2.0%	0.48	0.54	0.54
Retention time (%RSD)	<0.5%	0.13	0.10	0.07
Theoretical plates	>3000	8590	9116	9794
USP tailing	<2.0	1.12	1.13	1.19

[a] System suitability standard in diluted HCl (pH 1.2).

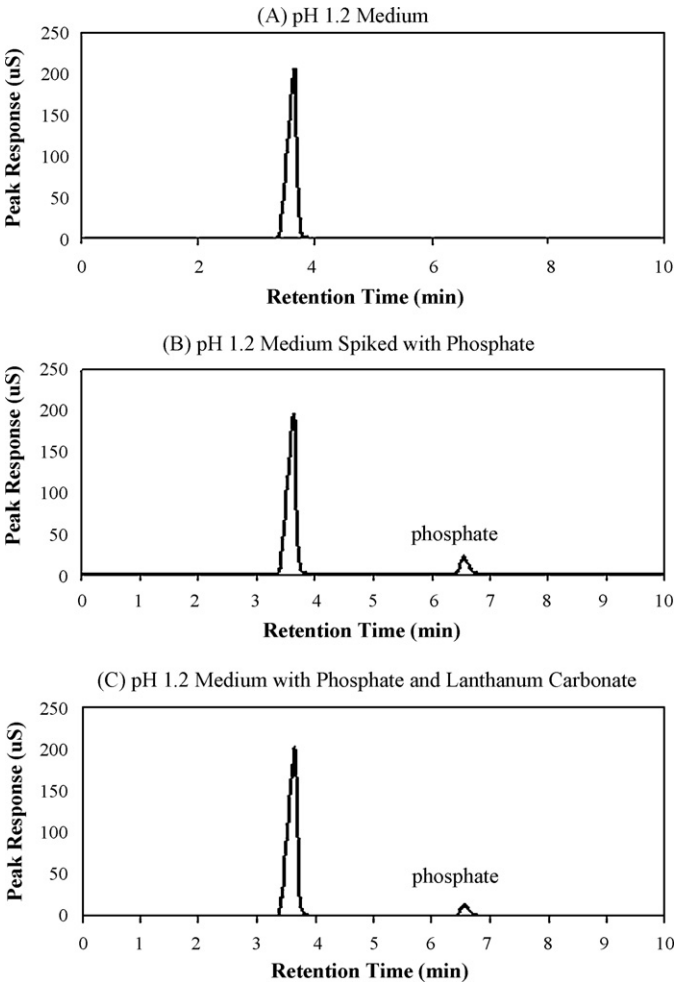

Fig. 1. Chromatograms of a pH 1.2 solution (A), the pH 1.2 medium spiked with phosphate standard (B), and the pH 1.2 solution containing both phosphate standard and lanthanum carbonate (C).

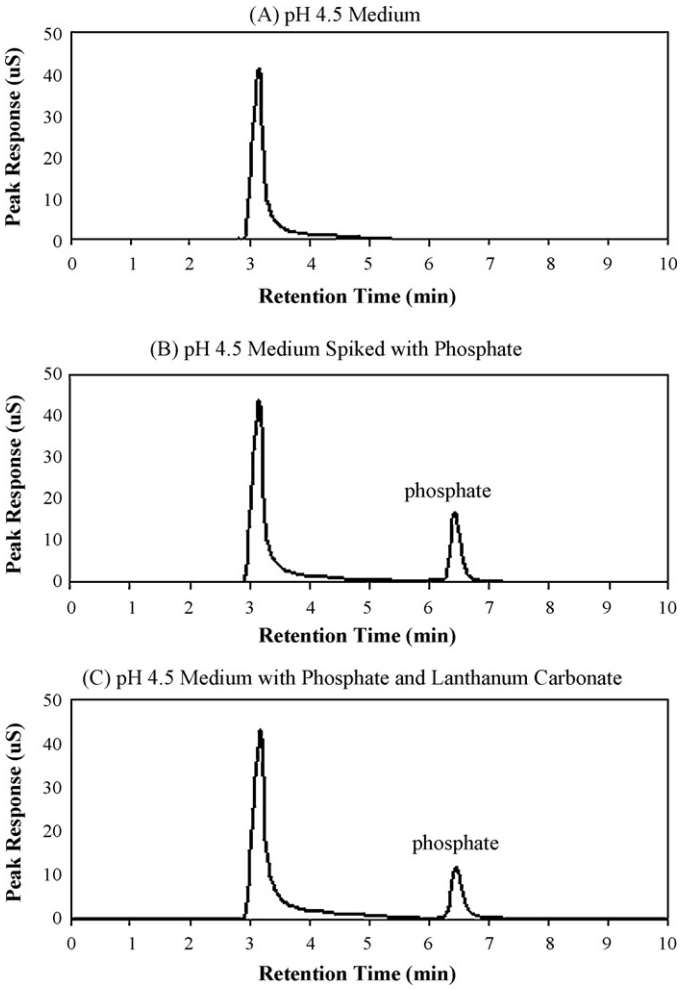

Fig. 2. Chromatograms of a pH 4.5 solution (A), the pH 4.5 medium spiked with phosphate standard (B), and the pH 4.5 solution containing both phosphate standard and lanthanum carbonate (C).

solutions at the phosphate concentrations of 2, 10, 50 and 200 ppm of the quality control standards. The mean precision ranged from 0.6% to 3.7% for three pH levels at the phosphate concentrations of 2, 10, 50 and 200 ppm of the quality control standards. Both accuracy and precision meet the guidance criteria [26]. The inter-day accuracy and precision of the method were studied by analyzing five identical samples containing 10, 50 and 200 ppm. The inter-day accuracy varied from 94% to 109%, while precision ranged from 1.2% to 2.1% for three pH solutions.

3.1.4. Limit of detection (LOD) and limit of quantification (LOQ)

The LOD is the lowest amount of drug that can be detected, but not necessarily quantitated based on a signal-to-noise ratio (S/N) of at least 6:1. The LOD was evaluated from five independent samples, which were spiked with phosphate in order to produce a S/N close the six times of the base line noise. Conversely, the LOD for all the testing conditions was estimated as 1 ppm. The LOQ is the lowest amount of analyte in the sample that can be determined with acceptable precision and accuracy. In our case, LOQ was determined as 2 ppm under the study conditions.

3.1.5. System suitability

System suitability standard solution containing 50 ppm of phosphate was prepared from a certified phosphate standard with appropriate pH media. The system suitability was determined from six replicate injections of the system suitability standard before sample analysis. The test is used to monitor critical operational parameters of the chromatographic system and to ensure the validity of the analytical procedure. All critical parameters tested met the acceptance criterion for three pH solutions on all days (Table 4).

3.1.6. Robustness

The robustness of the analytical method was evaluated by analyzing the system suitability standard and evaluating system suitability parameter data after varying, individually, the flow rate (±10%), injector volume (±50%), KOH eluent concentration (±1 mM) and column temperature (±5 °C) at the three different pH media. None of the variations were found to have caused any significant change in system suitability parameters namely capacity factor (k'), area (%RSD), retention time (%RSD), theoretical plates and USP tailing factor at all pH levels. Robustness data in diluted HCl (pH 1.2) are shown in (Tables 5–8). Similar results were obtained in pH 4.5 and pH 6.8 media.

3.1.7. Selectivity

To obtain the selectivity for phosphate and media components is a demanding task. The reason is not only that pH solutions contain high concentration of anions such as chlorine and borate but also carbonate released from lanthanum carbonate following ion exchange with phosphate. The chromatograms of pH 1.2 medium, phosphate spiked medium, and the medium containing both phosphate and lanthanum carbonate are shown in Fig. 1A–C.

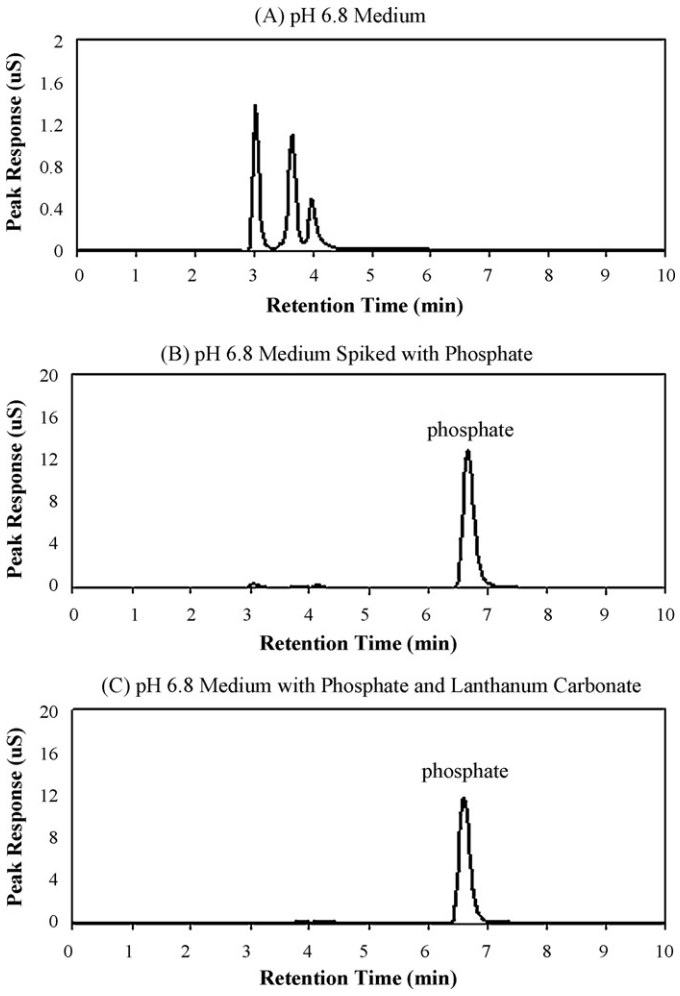

Fig. 3. Chromatograms of a pH 6.8 solution (A), the pH 6.8 medium spiked with phosphate standard (B), and the pH 6.8 solution containing both phosphate standard and lanthanum carbonate (C).

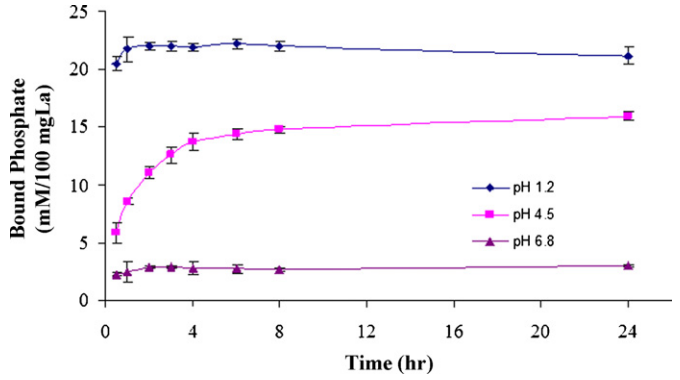

Fig. 4. The time-course of phosphate binding to lanthanum carbonate under three pH conditions. The initial concentration phosphate was 30 mM and the amount of lanthanum carbonate was 100 mg. The data represents the mean and standard deviation of triplicate samples.

The chromatograms of pH 4.5 medium, phosphate spiked medium, and the medium containing both phosphate and lanthanum carbonate are shown in Fig. 2A–C. The chromatograms of pH 6.8 medium, phosphate spiked medium, and the medium containing both phosphate and lanthanum carbonate are shown in Fig. 3A–C. Note: the 3 peaks seen in Fig. 3A are from borate ions in the blank sample of pH 6.8 buffer. Phosphate and other component such as chloride peaks in these chromatograms are well resolved from each other.

3.2. Applications

The validated method was applied to determine the phosphate binding capacity for both drug product and drug substance of lanthanum carbonate under three different pH conditions (pH 1.2–6.8), over a incubation period of 0.5–24 h. A typical phosphate binding time-course profile for the lanthanum carbonate drug substance is shown in Fig. 4. The data suggest that the phosphate binding equilibrium was reached at 8 h. Therefore, the binding data at 8 h can be used for calculation of the binding parameter constants.

4. Conclusion

A simple and efficient IC method was developed and validated over human gastrointestinal pH conditions in our study. The method addressed each of the analytical validation characteristics such as linearity, accuracy, precision, stability, robustness and selectivity, and met the acceptance criteria defined in the guidance. The usefulness of this method is demonstrated by successful application for the *in vitro* determination of phosphate binding to lanthanum carbonate under the human gastrointestinal pH conditions at different time-points that reflect gastrointestinal residence time.

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