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Eco-friendly RP-HPLC UV Quantification Method for Parental Products Buffer Agents by Utilizing Polymethacrylate Resin Stationary Phase in Light of Quality by Design

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Parental products buffer agents *viz*. calcium phosphate (CP), potassium phosphate (PP) and sodium phosphate (SP) were successfully qualitatively and quantitatively determined on RP-HPLC in the UV detection. The three components were retained in reverse phase by utilizing polymethacrylate resin stationary phase. The quantification was done by using the waters IC-Pak (50 × 4.6) mm, 10 µm column with 1.0 mL/min flow rate. 0.1 M nitric acid solution was used as a mobile phase. The detection was done at 288 nm, column temperature was 30 ºC, injection volume was 50 µL and the total run time was 5 min. The method was validated as per the regulatory and ICH guidelines. The accuracy was found from 98.5% to 101.5% and the precision data was found from 0.5% to 0.9%. The linearity resulted in r value more than 0.999. Quality by design software was used to establish the method robustness. Green chemistry principles and tools were used to assess the method eco-ness. The developed method can be utilized as an application to quantify the buffer agent content in selected parental products.

Keywords: Calcium phosphate, Potassium phosphate, Sodium phosphate, QbD, Analytical eco-scale.

INTRODUCTION

It is important to select the right buffer during the formulation process, because protein solubility as well as physical and chemical stability are pH dependent. In parental preparations, pH must be close to physiologic levels in order to optimize drug solubility and stability [1]. The addition of buffers to a formulation allows the pH to be maintained at an optimal level, which in turn increases the solubility and stability of the medicine [2]. The pH values for injectable products should range from 3.0 to 9.0 before administration. Injecting a solution with a pH outside this range might injure nearby tissues [3]. Typical buffer systems used in biotech formulations include phosphate, citrate and acetate. Citric acid, sodium citrate, sodium acetate, mono and dibasic sodium phosphate and other buffer components are frequently employed in parent goods. Citric acid, sodium citrate, sodium acetate can be easily determined by using the HPLC UV detection because due to the acetate and citrate anions, three compounds show the UV index. Phosphate buffer agents are not showing the UV index due to the lack of chromophore. Calcium phosphate (CP), potassium phosphate (PP) and sodium phosphate (SP) are the regular excipients and used as buffering agents in the parental products [4].

Calcium phosphate is a naturally occurring mineral found in abundance in teeth and bone. The compound serves multiple functions in the body and supplementation may be useful for some people. There are, however, potential side effects from consuming too much calcium phosphate [5]. The circulatory system and tissues also contain calcium. Trusted source and protein and DNA both contain phosphorus trusted source [6]. These minerals are crucial for the health of the bones, muscles, blood and nerves.

Human body needs phosphorus, a naturally occurring substance, in every cell. All body cells contain phosphorus, which is necessary for tissue growth and repair [7]. Hypophosphatemia (low levels of phosphorus in the blood) is treated or

prevented with potassium phosphate. Intravenous fluids given to patients who are unable to swallow may contain potassium phosphate [8].

According to the US Food and Drug Administration (FDA), sodium phosphate is a component used in the manufacturing goods as well as a number of pharmaceuticals and everyday things. Some people's bowels may be prepared for a colonoscopy using sodium phosphate [9]. A saline laxative called sodium phosphate is thought to work by raising the amount of fluid in the small intestine. Between 30 min and 6 h later, it typically causes a bowel movement. If not prescribed by a doctor, do not administer this medication to children under the age of five [10].

Calcium phosphate (CP), potassium phosphate (PP) and sodium phosphate (SP) do not have the chromophore, thus leading to quantification by using HPLC with UV detection is not possible. But due to the presence of phosphate anion, it was possible to determine CP, PP and SP by the RP-HPLC UV method. For the quantification, meanwhile, a variety of analytical approaches and techniques are available in the literature [11-16]. However, it is necessary to perform the derivatization in order to determine the CP, PP and PP in the UV mode using potassium phthalate.

Quality by design (QbD) is a statistical tool that has been used in conjunction with design of experiments (DoEs) [17-21] in order to improve the critical quality metrics of the method. The investigation was carried out using design expert program and proposed the environmental friendliness of the procedure, which was calculated using the analytical eco-scale [21-24].

EXPERIMENTAL

Excipient grades *viz*. calcium phosphate (CP), potassium phosphate (PP) and sodium phosphate (SP) were purchased from Merck Ltd, China. Nitric acid, potassium hydrogen phthalate (PHT) analytical grade reagents were brought from SinoPharm Chemical Reagents, China. Class 'A' glasswares and in-house Milli-Q water were used in the research.

The chromatographic data was acquired with Empower software. Waters HPLC with a PDA/UV detector was utilized for chromatographic analysis. Sartorius analytical balances (models: CPA255D and CP2P) were used for solution preparation. The chromatographic columns such as Ultracarb C8 (150 × 4.6) mm, 5 mm column from Phenomenex and IC-Pak Anion (50 × 4.6) mm, 10 µm from waters were procured for the method development, validation and regular analysis.

Method: The mobile phase was made by adding 5 mL of 0.1 M nitric acid, mixed well and diluted it to 1000 mL with water after adding 200 mg of potassium hydrogen phthalate (PHT) to 100 mL of water and mixed well. An IC-Pak Anion (50 mm × 4.6 mm, 10 µm) HPLC column with a flow rate of 1.0 mL/min, a column temperature of 30 ºC and an injection volume of 50 µL was employed with a UV detector-equipped chromatography system. At the wavelength of 288 nm, the detection was carried out and the entire runtime was 5 min. Water (diluent) with a 15 µg/mL concentration was used to make the standard and sample solutions. The calculated amount of disodium phosphate (DSP) present in the samples after injecting them into HPLC.

RESULTS AND DISCUSSION

Optimization of chromatographic method: Calcium phosphate (CP), potassium phosphate (PP) and sodium phosphate (SP) cannot be quantified by HPLC because it lacks a chromophore. The initial chromatography conditions were started with an IC-Pak (50 mm × 4.6 mm, 10 m) column based on the literature. Polymethacrylate resin with a quaternary ammonium functional group is present in the column material. The column material interacts with the anion (HPO_4^{2-}) and adsorbs it. The choice of mobile was crucial because CP, PP and SP, an anion (HPO_4^{2-}) of all the three compounds, these anions will adsorb on the column material. Ordinary phosphate buffers and other anionic buffers are not suitable for elution because they interfere with CP, PP and SP. In this case, the mobile phase should consist of an appropriate ion-interaction reagent in an aqueous solution to increase the adsorption capability of compounds. To increase the mobile phase's background absorption, potassium hydrogen phthalate (PHT) was added as an ion-interaction reagent. As a result, the anion will be retained in the reverse phase and the lipophilicity will increase. The mobile phase was prepared by adding PHT to a variety of buffers, including 0.1 mM nitric acid buffer, 1.3 mM boric acid buffer and 1.3 mM gluconic acid buffer. The CP, PP and SP sample solutions were injected into the HPLC at a flow rate of 1.0 mL/min in 50 µL volume. The gluconic acid and boric acid buffers showed no peak, while the peak was eluted with a distorted peak shape, which was phosphate anion eluted in the nitric acid buffer. The peak UV spectrum was recorded and selected the wavelength 288 nm for further optimization, the UV spectrum is shown in Fig. 1. The nitric acid buffer concentration was raised from 0.1 mM to 0.1 M to enhance the peak shape. The PHT concentrations of 0.5 mM, 1.0 mM, 1.5 mM and 2.0 mM were examined to determine the PHT concentration that would work best in the mobile phase. The CP (calcium phosphate), PP (potassium phosphate) and SP (sodium phosphate) peaks were used for further analysis since it satisfied the system suitability requirements with a concentration of 1.0 mM PHT. No interference between the diluent and placebo samples was seen at the monitoring wavelength of 288 nm. The final, interference-free and optimized chromatograms is shown in Fig. 2.

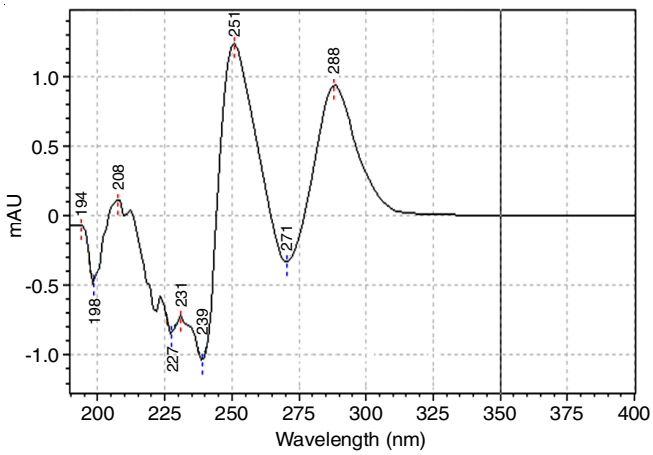

Fig. 1. UV spectrum of analyte

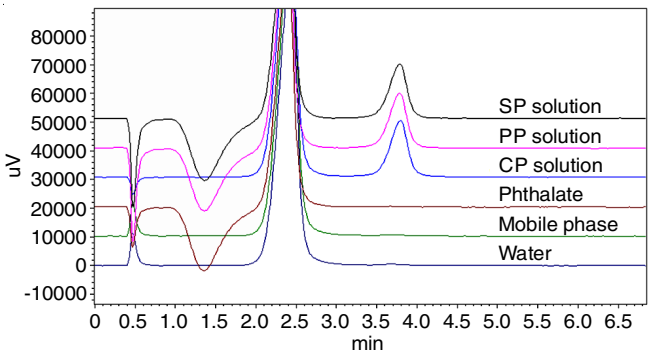

Fig. 2. Final chromatogram of calcium phosphate (CP), potassium phosphate (PP) and sodium phosphate (SP) overlaid with blank, mobile phase and phthalate solution

Analytical method validation: The developed method was validated as per the regulator guidelines, which are USP 621, USP 1225, EP 2.2.24 and ICH Q2<R1>. The parameters considered for the validation were mentioned in the below points [25-28].

Specificity: The method specificity was proven by injecting the blank (diluent), mobile phase buffer and standard solution. The chromatographic system ran in PDA mode to check the purity of peak (Fig. 3). The developed method resulted in no interference observed from diluent and mobile phase. The system suitability and specificity results are mentioned in Table-1.

Linearity: The method's linearity was tested using different concentrations ranging from 0.3 µg/mL to 80.0 µg/mL concentration range. The obtained results are linear, which express that the method obeys the beer- Lambert's law.

Accuracy: The recovery study conducted by spiking the known amount of CP, PP and SP. The spiked sample quantified in the developed method and recoveries obtained from 98.5% to 101.5%. The results mentioned in Table-1 confirmed that the method was accurate.

Repeatability and Intermediate precision: The method precision was demonstrated by preparing six samples of CP, PP and SP from a single batch at concentration of 50 µg/mL. Quantified the recovery of six samples and the % RSD of six recovery samples was found to be 0.5% for CP, 0.9% for PP and 0.9% for SP.

The intermediate precision of the developed method was evaluated by preparing six samples of CP, PP and SP from a single batch at concentration of 50 µg/mL in a different day by using different lot of columns and different HPLC. Quantified

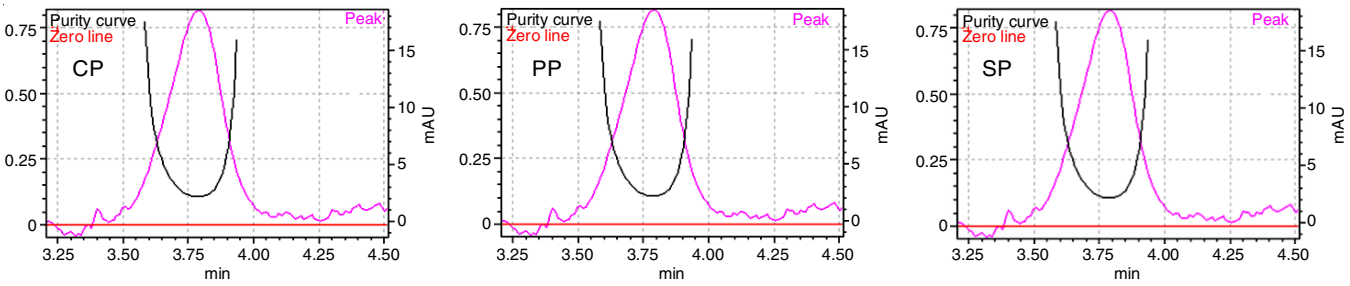

Fig. 3. Peak purity plots of calcium phosphate (CP), potassium phosphate (PP) and sodium phosphate (SP)

TABLE-1
ANALYTICAL METHOD VALIDATION DATA FOR CALCIUM PHOSPHATE, POTASSIUM PHOSPHATE AND SODIUM PHOSPHATE

Parameter name	Calcium phosphate	Potassium phosphate	Sodium phosphate
System suitability			
Tailing factor (< 2.0)	1.3	1.4	1.3
Plate count (> 2000)	4596	4929	4893
% RSD (n = 6 < 2.0)	0.5	1.1	1.0
Specificity			
Diluent/mobile phase interference (should be absent)	No interference	No interference	No interference
Peak purity (should be passed)	Passed	Passed	Passed
Linearity			
Range (µg/mL)	0.3-80.0	0.3-80.0	0.3-80.0
Slope	6196	6965	6473
Intercept	3196.6	3295.9	3365.8
Correlation coefficient > 0.999	0.9996	0.9991	0.9992
Accuracy (n = 3 avg. percentage)			
80% mean ± SD	101.1 ± 0.5	100.8 ± 1.3	98.6 ± 1.2
100% mean ± SD	98.5 ± 1.1	101.5 ±1.4	101.5 ± 1.2
120% mean ± SD	100.5 ± 1.0	100.9 ± 1.6	99.4 ± 1.1
Precision (n = 6% RSD < 2.0)	0.5	0.9	0.9
Intermediate precision (n = 6% RSD < 2.0)	0.9	0.5	0.6
Ruggedness (n = 12% RSD < 2.0)	0.7	0.7	0.8
Solution stability B.T (0 & 24 h % difference < 2.0)	1.1	1.2	1.5
Solution stability 2-8 °C (0 & 24 h % difference < 2.0)	1.2	0.4	1.6
Solution stability B.T (0 & 48 h % difference < 2.0)	0.8	1.6	1.0
Solution stability 2-8 °C (0 & 48 h% difference < 2.0)	1.9	1.6	0.4

the recovery of six samples and the % RSD of six recovery samples was found to be 0.9% for CP, 0.5% for PP and 0.6% for SP.

Solution stability: The stability of the solutions in the selected diluent for using longer times done the evaluation experiment. The standard solutions of CP, PP and SP were injected immediately in the optimized method and stored solution at bench top and refrigerator conditions. The stored bench top and refrigerator samples were injected again at 24 and 48 h. The % difference found below 2.0% and solutions were found to be stable for 48 h (Table-1).

Robustness: The method robustness was investigated by employing the quality by design tool to assess method performance. The optimized method critical method parameters (CMP) were pointed and used for the study. The flow rate was changed to ± 10%, nitric acid strength changed to ± 10% and column temperature changed to ± 5 ºC. The full factorial design was constructed by using the three factors with three-center points with zero blocks. A total of 19 trials were performed with the HPLC and the design expert software, with the retention time (R1), tailing factor (R2), and plate count (R3) serving as the replies (Table-2). The ANOVA table (Table-3) shows that the

TABLE 2
QUALITY BY DESIGN TOOL EVALUATED DESIGN OF EXPERIMENTS DATA

Std.	Run	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
		A: Flow	B: Nitric acid strength	C: Column temp.	Retention time	Tailing	Plate count
3	1	1.1	0.9	25	2.991	0.991	2569
10	2	0.9	0.9	35	4.459	0.851	5698
6	3	0.9	1.1	25	4.692	1.156	5196
11	4	1.1	0.9	35	3.395	0.995	2998
1	5	0.9	0.9	25	2.961	0.989	5129
17	6	1	1	30	3.651	1.051	4561
5	7	0.9	1.1	25	4.451	0.995	5691
9	8	0.9	0.9	35	4.51	0.889	4998
14	9	0.9	1.1	35	4.659	0.981	4892
7	10	1.1	1.1	25	4.651	1.216	3165
16	11	1.1	1.1	35	3.995	1.215	3269
4	12	1.1	0.9	25	3.962	0.995	3561
12	13	1.1	0.9	35	3.985	1.125	3297
8	14	1.1	1.1	25	3.996	1.109	3671
18	15	1	1	30	3.666	1.02	4610
19	16	1	1	30	3.66	1.06	4597
15	17	1.1	1.1	35	2.985	1.095	3629
13	18	0.9	1.1	35	4.671	1.05	5261
2	19	0.9	0.9	25	4.661	0.99	4987

TABLE-3
ANALYSIS OF VARIANCE (ANOVA) TABLE

Response	Source	Sum of squares	df	Mean square	F-value	p-value	
R1	Model	1.63	1	1.63	5.54	0.0317	Significant
	A-Flow	1.63	1	1.63	5.54	0.0317	
	Curvature	0.4144	1	0.4144	1.41	0.2523	
	Residual	4.7	16	0.2938			
	Lack of Fit	1.85	6	0.3091	1.09	0.4317	Not significant
	Pure Error	2.85	10	0.2846			
	Cor Total	6.74	18				
R2	Model	0.1235	4	0.0309	9.3	0.0009	Significant
	A-Flow	0.0441	1	0.0441	13.29	0.003	
	B-Nitric acid strength	0.0615	1	0.0615	18.53	0.0009	
	C-Column temp.	0.0036	1	0.0036	1.08	0.3166	
	AC	0.0143	1	0.0143	4.3	0.0585	
	Curvature	0	1	0	0.0095	0.9236	
	Residual	0.0431	13	0.0033			
	Lack of Fit	0.0048	3	0.0016	0.419	0.7434	Not significant
	Pure Error	0.0383	10	0.0038			
	Cor Total	0.1667	18				
R3	Model	1.54×10^7	1	1.54×10^7	151.67	< 0.0001	Significant
	A-Flow	1.54×10^7	1	1.54×10^7	151.67	< 0.0001	
	Curvature	2.90×10^5	1	2.90×10^5	2.85	0.1105	
	Residual	1.62×10^6	16	1.02×10^5			
	Lack of Fit	4.47×10^5	6	74539.9	0.6336	0.702	Not significant
	Pure Error	1.18×10^6	10	1.18×10^5			
	Cor Total	1.73×10^7	18				

evaluated design for 3 factors are adequate and suitable. The Pareto charts, 2D half normal plots, 2D contour plots are shown in Fig. 4, while the 3D surface plots, overlay plots and 3D cube plots for responses are shown in Fig. 5. The results indicated that the tailing factor was impacted by nitric acid strength and plate count was affected by flow rate but the variations are within the limit and the method was found to be robust.

Method eco-friendliness assessment: The method eco-friendliness was assessed by calculating the penalty points for the method. The penalty points calculated for solutions, sample preparations, instruments, energy and waste for each step are mentioned in Table-4, which indicated that the method is eco-friendly.

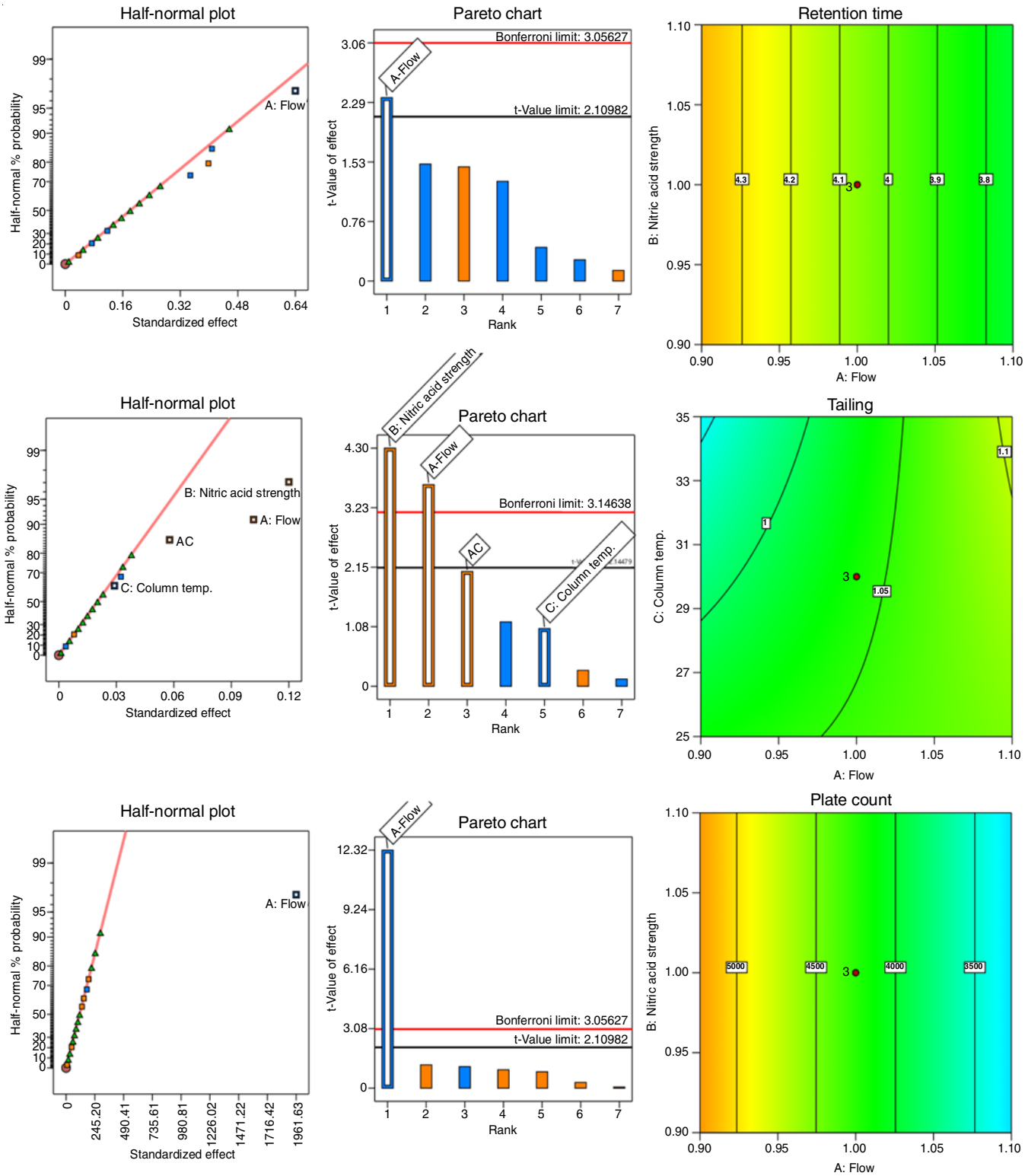

Fig. 4. Half normal plots, Pareto charts and 2D contour plots for response R1, R2 and R3

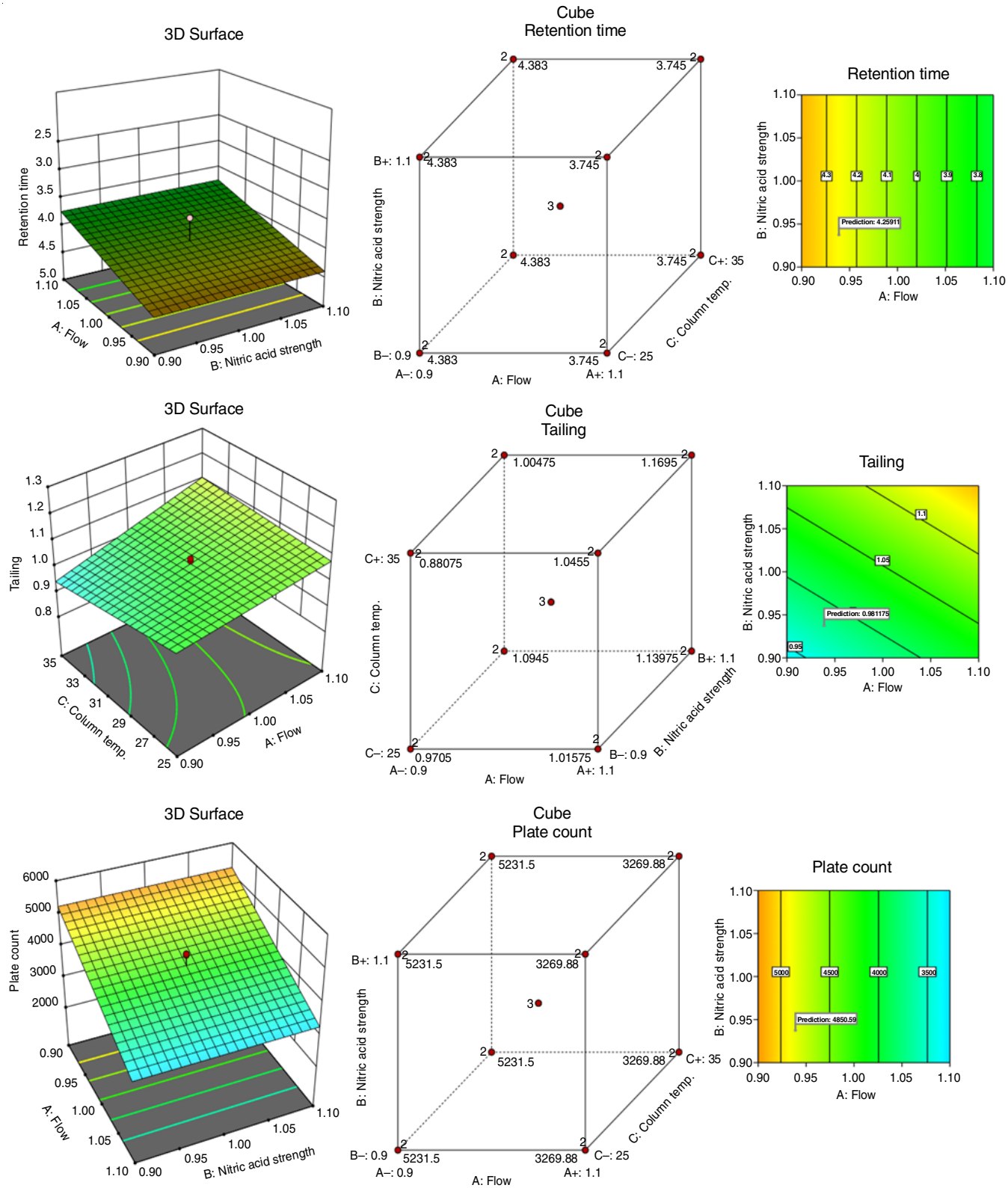

Fig. 5. 3D surface plots, 3D cube and overlay plots for responses R1, R2 and R3

Conclusion

An eco-friendly RP-HPLC UV quantification method for the parental products buffer agents calcium phosphate (CP), potassium phosphate (PP) and sodium phosphate (SP) using polymethacrylate resin stationary phase was successfully developed and verified in accordance with regulatory criteria. The method was evaluated by using the modern statistical tools quality by design and proved the method is robust, no impact of small variations in the method conditions. The analytical eco-scale value 90 shows that the proposed method was eco-friendly.

TABLE-4
PENALTY POINTS FOR THE
PROPOSED DEVELOPED METHOD

Reagents/instruments	Penalty points
Nitric acid	4
Potassium hydrogen phthalate	0
HPLC	1
Occupational hazard	0
Waste	5
Total penalty points	10
Analytical eco-scale	Σ 90

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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