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Letter

Determination of phosphorous oxoanions in pharmaceuticals using non-suppressed ion chromatography

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Abstract. The separation and quantitation of phosphorous oxoanions have been investigated by non-suppressed ion chromatography (IC) with conductometric detection using a modified gluconate-borate buffer as eluent. Hypophosphite being coeluted with the injection peak, its previous oxidation into phosphate is necessary. The method was applied to the determination of hypophosphite and phosphate in several pharmaceuticals.

Key words. Phosphate determination – hypophosphite determination – pharmaceuticals – non-suppressed ion chromatography – conductometric detection.

Introduction

Phosphorous oxoanions are present in pharmaceutical formulations due to two sources: as active ingredients (orthophosphate and hypophosphite) or as salt forming agents, counter-ions of several organic active ingredients. In the first case, orthophosphate is used in hypercalcemia and as urinary acidificant. Hypophosphites, apart from its former use in the treatment of tuberculosis, are used as anabolyzing agents, by favouring the biosynthesis of proteins. In the second case, there are several therapeutic salts involving phosphate as counter-ion such as, for instance, codeine phosphate, pyridoxal phosphate or dexamethasone phosphate.

Phosphonoformate is a potent antivirus substance which produces, as degradation products phosphite and phosphate; it requires suitable analytical methods for its monitoring in biological fluids [1].

Phosphorous oxoanions, when present as minor or trace level, are currently determined spectrophotometrically, preferably as molybdenum blue or using the phosphovanadomolybdate method [2]. Phosphate is directly determined. Hypophosphite and phosphite are oxidized to phosphate prior their quantitation [2]. This standard procedure is tiresome compared with some recent procedures based on ion chromatography (IC). Tanaka et al. [3] and Biesaga and Trojanowicz [4] determined phosphorous oxoanions in plating solutions using the suppressed IC. Ryder [5] described the separation of phosphorous oxoanions by using single-column IC with conductivity detector employing succinic acid (pH 3) as eluent. Hatton and Pickering [6] analized phosphorous oxoanions and other inorganic anions using p-hydroxybenzoic acid or p-aminobenzoic acid as suitable eluents and refractive index detection. Schmuckler et al. [7] proposed the use of gluconate-borate eluent for anion chromatography, which is actually, the eluent most widely used in non-suppressed or single column IC. Moreover, Waters Corporation commercializes a borate-gluconate type eluent for these purposes [8]. Ruiz et al. [9] used this eluent to determine inorganic water-soluble phosphate in vegetables. Bello and González [10] have been adapted the borate-gluconate procedure to determine phosphate in cola beverages.

The aim of the present paper is the study of separation and quantitation of hypophosphite, phosphite and phosphate by non suppressed IC with conductometric detection and its application to the determination of phosphorous oxoanions in drug formulations. The proposed procedure is fast, feasible, accurate and favourably compares with the time-consuming spectrophotometric methods. However, the price of the apparatus is much higher. Anyway, taking into account that standard HPLC systems are today available in the majority of research labs, the only extra requirements to apply our procedure will be an IC column and a conductometric detector.

Materials and methods

Chromatographic system

Equipment

A Waters 501 HPLC pump was used together with a Waters IC-Pak A HR packed with polymetacrylate resin with a quaternary ammonium functional group (6 μm particle size and

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exchange capacity of 30 ± 3 μeq mL^{-1}) fitted with a Waters Guard-Pak precolumn. Samples were injected by using a Rheodyne type injector with a 100 μL loop. Detection was done from a Waters 341 Conductivity detector. Peak evaluations were done with an Hewlett Packard HP3395 integrator.

Chromatographic conditions

Eluent preparation: Prepare the concentrate solution A by dissolving 16 g of sodium gluconate, 18 g of boric acid and 25 g of sodium tetraborate in a mixture 25:75 v/v of glycerol:water to 1 L.

The working eluent is prepared by mixing 12 mL of concentrate solution A, 20 mL of *n*-butanol, 120 mL of acetonitrile and water up to 1 litre (pH 8.4). This eluent was degassed and micro-filtered (0.2 μm) before use. Its conductivity was of about 180 μS (% Full Scale).

The flow rate was 1 mL min^{-1}. The detector operates at a Base range of 200 μS and the integrator was used with an attenuation of 128.

Reagents

Potassium permanganate (Merck), Potassium phosphate (Merck), phosphorous acid (Aldrich), sodium hypophosphite monohydrate (Fluka), boric acid (Merck), sodium gluconate (Fluka), sodium tetraborate decahydrate (Riedel), glycerol, n-butanol and acetonitrile (Merck) were of analytical reagent grade or better. Milli-Q treated water was used throughout.

Stock solutions of phosphate, phosphite and hypophosphite of 1000 mg L^{-1} were prepared and standardized if necessary.

For the sample mineralization, concentrated nitric acid and 30% hydrogen peroxide (Merck) were utilized.

Samples

Phosphate was determined in three antitusive formulations based on codeine phosphate (INISTON ANTITUSIVO®, (Gayoso Wellcome), CODEISAN®, (Abelló) and FIORINAL CODEINA®, (Sandoz Pharma)). Hypophosphite was determined in three nutritional restoratives (CALCIOGEVE®, (Bama-Geve), OSVICAL®, and OSVICAL-LISINA®, (Alter)). The composition of each formulation is given in the *Appendix*.

Procedures

Sample treatments

Samples containing phosphate were alkalized (pH 8.5–9), diluted and passed through a Waters C_{18} SEP-Pak Plus cartridge (short body) before injection. Samples containing hypophosphite were digested with concentrated nitric acid and hydrogen peroxide in a sand bath near to dryness. Diluted portions were directly injected.

Calibration features

The chromatographic signal used in calibrations was the height peak ratio (with respect to the internal standard) according their reliability, better than peak area ratio.

A series (n = 10) of standard solutions (four replicates) of phosphorous oxoanions were prepared and measured. Data about the calibration graphs are collected in table I. Hypophosphite coelutes with the injection peak and a derivatization prior injection is required. So, standard solutions of hypophosphite were oxidized into phosphate with permanganate 0.5 M in acidic medium [2]. The excess of permanganate was removed with 3% hydrogen peroxide.

The application of Student's t-test shows that the intercepts are insignificant and accordingly, the straight lines passe through the origin.

Results and discussion

The separation of hypophosphite, phosphite and phosphate, in the proposed mobile phase shows good resolution (Fig. 1). The species involved in the separation at the pH of the mobile phase were: $H_2PO_2^-$, HPO_3^{-2} and HPO_4^{2-}. One characteristic of the non-suppressed IC with conductivity detection is the appearance of an injection peak; this is due to the displacement of the eluent ions by the injected ions [11]. Unfortunately, the peak corresponding to hypophosphite is very close to the injection peak, as can be seen in figure 1, and so, it coelutes with it. Accordingly, hypophosphite cannot be accurately quantitated directly from the chromatogram. It is more advisable to perform a derivatization into phosphate prior injection. If the sample contains both phosphate and hypophosphite, two injections are needed: with and without derivatization.

Table I. Calibration data.

Analyte	*Regression parameters*	*LCR*[a]	*r*[b]	*LOD*[c]
Phosphate	$y = (0.012 \pm 0.006) + (0.098 \pm 0.001)x$	0.5–10	0.9994	0.2
Phosphite	$y = (0.022 \pm 0.006) + (0.176 \pm 0.001)x$	0.5–8	0.9990	0.1
Hypophosphite[d]	$y = (0.01 \pm 0.01) + (0.103 \pm 0.002)x$	0.5–10	0.9986	0.3

[a] Linear concentration range (mg L^{-1}); [b] Correlation coefficient; [c] Detection limit (mg L^{-1}); [d] After derivatization (see text).

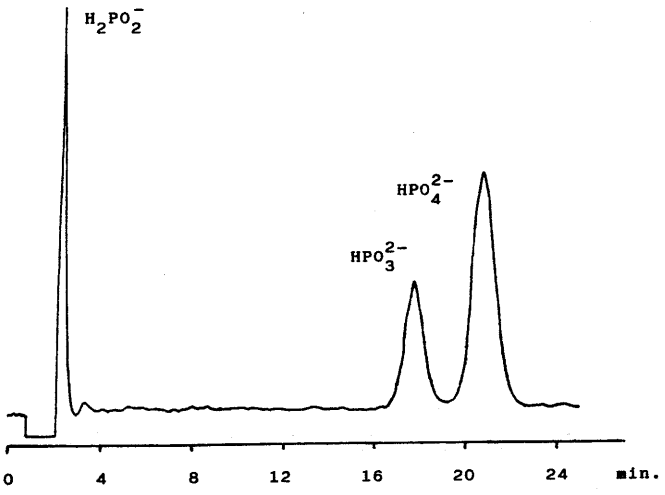

Fig. 1. Separation of phosphorous oxoanions by the described procedure. Injection of 100 µl containing 5 mg L^{-1} of each oxoanion.

Tanking into account that (apart from its appearance as a metabolite of phosphonoformate) phosphite is scarcely found in pharmaceutical formulations, the proposed procedure was applied only to samples containing phosphate or hypophosphite. The results obtained as percentage of the content of active ingredient claimed in the label for both the proposed method and a spectrophotometric method [2] are presented in table II. As can be observed, good agreement were found between both procedures. This was statistically proved according to the paired t-test [12] and also by considering the label claimed for analyzed samples, within the confidence limits accepted by pharmacopoeias.

Conclusions

The proposed IC method for determining phosphorous oxoanions is faster and easier than the conventional spectrophotometric procedures; however, investment is higher. The necessity of hypophosphite derivatization is not a drawback because in mixtures, the spectrophotometric procedure requires the conversion of any phosphorous oxoanion into phosphate. The proposed IC method applied to several pharmaceutical formulations gives good results.

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Appendix

Composition of the analyzed samples

CODEISAN® (contents for 15 mL of syrup)

Codeine phosphate hemihydrate	19 mg
Ephedrine hydrochloride	30 mg
Sodium Benzoate	150 mg
Fluid extract of primrose	100 mg
Flavoured syrup, excipient up to 15 mL	

INISTON ANTITUSIVO® (contents for 5 mL of syrup)

Codeine phosphate hemihydrate	10.60 mg
Pseudoephedrine hydrochloride	28.25 mg
Tripolidine hydrochloride	1.40 mg
Sucrose (excipient)	3.5 g

FORINAL CODEINA® (content for one capsule)

Codeine phosphate hemihydrate	14.67 mg
Caffeine anhydrous	40 mg
Acetaminophen	300 mg
Acetylsalicylic acid	200 mg

CALCIO GEVE® (contents for one effervescent tablet)

Calcium hypophosphite	100 mg
Calcium levulinlactogluconate	500 mg

Table II. Percentage of the label claimed for the analysed samples obtained from the proposed method and from spectrophotometric methods (see text).

SAMPLE	*HYPOPHOSPHITE*		*PHOSPHATE*	
	Proposed method	*Spectrophotometric method*	*Proposed method*	*Spectrophotometric method*
Codeisán®	—	—	97.5%	98.2%
Inistón Antitusivo®	—	—	96.2%	97.1%
Fiorinal Codeina®	—	—	98.4%	98.1%
Calcio Geve®	99.5%	99.3%	—	—
Osvical®	101.7%	100.9 %	—	—
Osvical Lisina®	98.8%	99.3 %	—	—

Calcium heptagluconate	500 mg
Vitamin C	1000 mg
Vitamin D_3	3000 IU
Sodium benzosulphimide	7 mg

OSVICAL® (contents for one effervescent tablet)

Calcium hypophosphite	686 mg
Calcium levulinate-carbonate	846 mg
Vitamin C	500 mg
Cholecalciferol	100 IU
Saccharine	60 mg

OSVICAL LISINA® (contents for one tablet)

Calcium hypophosphite	300.00 mg
Calcium ascorbate	560.00 mg
Calcium pantoneate	5.00 mg
Carnitine hydrochloride	200.00 mg
Cobamamide	0.15 mg
Ergocalciferol	12.50 µg
Lisine hydrochloride	250.00 mg
Calcium adipate	50.00 mg
Calcium carbonate	100.00 mg
Calcium citrate	1315.00 mg.

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