Eurasian Journal of Analytical Chemistry
Volume 3, Number 2, 2008

Liquid Chromatography with Indirect UV Detection for Determination of Some Inorganic Anions Using a Permanently Coated Column: Determination of Monofluorophosphate, Phosphate and Iodide

Mohamed Salem Rizk, Safaa Shafik Toubar and Aliaa Ibrahim Shallan
Helwan University, Faculty of Pharmacy, Analytical Chemistry Department, Cairo, Egypt.

Abstract

A method for the separation and determination of inorganic anions using a reversed phase column permanently coated with *n*-cetylpyridinium chloride is described. A 1 mM sodium salicylate solution in 5% (v/v) methanol was used as the mobile phase. The separation of some inorganic anions (monofluorophosphate (MFP), phosphate and iodide) was carried out in about 10 minutes. Detection was made using indirect UV detection at 290 nm. Satisfactory reproducibility of the results was obtained even after a four months period of continual use of the column. Through the evaluation of the analytical parameters, the method was linear (r = 0.999) at concentrations ranging from 10.0 to 100.0 µg mL-1.

Keywords: Cetylpyridinium chloride, Inorganic anions, Ion chromatography, Permanently coated column.

1. Introduction

Inorganic anions have been identified and determined in pharmaceutical materials using different techniques. They may be present as minor components or impurities. Different Pharmacopoeias specify limit tests to be performed before accepting a pharmaceutical material. Presence of interfering substances and the low concentration of the anions to be determined may complicate the analysis. In most cases, separation and pre-concentration steps are required before starting the analysis.

Ion chromatography seems to be the most widely used technique for determination of inorganic anions. The use of reversed-phase columns that are dynamically or permanently coated to give an ion-exchange behavior have been reported. Columns can be coated with hexadecyltrimethyl ammonium [1 – 4], cetyltrimethyl ammonium [5 – 10], hexadecylpyridinium [11] or *n*-cetylpyridinium [12 – 14]. Elution using an UV-absorbing mobile phase enables indirect UV detection [2 – 4, 7, 8, 12 – 14].

ISSN: 1306-3057

The aim of this study was to develop and validate a simple and fast liquid chromatographic method, through evaluation of the parameters of linearity, precision, accuracy, detection and quantitation limits and specificity for direct determination of MFP, phosphate and iodide. The method was proved suitable for the determination of MFP in toothpastes and trace amounts of phosphate and iodide in pharmaceutical raw materials.

2. Experimental

A Gilson liquid chromatograph equipped with a 321 Gilson pump and a 155 UV-Vis. Gilson detector set at 290 nm was used. The stationary phase was a Waters® C_{18} Spherisorb ODS2 column (150 × 4.6 mm, 5 µm particle size).The column was permanently coated with *n*-cetylpyridinium chloride according to the method described by Jun et al. [12]. The mobile phase was 1 mM sodium salicylate solution in 5% (v/v) methanol. The injection volume was 20 µL, and the run time was 10 minutes. The mobile phase was prepared daily, filtered through a 0.45 µm membrane filter (Millipore, Milford, MA) and degassed in an ultrasonic bath for 15 minutes. The mobile phase flow rate was 2.0 mL min^{-1}. Analytical data was stored in a computer equipped with Unipoint software and connected to the chromatographic system by a Gilson system interface.

All chemical were analytical reagent grade and were used as received. Sodium monofluorophosphate (MFP, Na_2PFO_3), sodium dihydrogen phosphate ($NaH_2PO_4.2H_2O$), potassium iodide (KI), *n*-cetylpyridinium chloride as its monohydrate and sodium salicylate were purchased from Merck (Darmstadt, Germany). Methanol (Riedel-deHäen, Sleeze, Germany) HPLC grade was used. All solutions were prepared in double distilled water.

2.1. Calibration curves.

The aqueous stock reference solution was prepared to contain 0.1% g (w/v) of the anion to be determined. Appropriate dilutions were made to give final concentrations in the range 10.0 to 100.0 µg mL^{-1}. The solutions were filtered through a disposable syringe filter before column injection. Each solution was injected in triplicate. The chromatograms for the determined anions are shown in Figures 1 (a – d). The absolute peak area in each case was plotted as a function of anion concentration to determine the quantitative range for each anion.

2.2. Application procedures.

2.2.1. Determination of MFP in toothpaste

Accurately weighed amount, by difference, of toothpaste (approximately 0.50 g) was sonicated for 30 minutes in 100 mL double distilled water, followed by centrifugation for 10

minutes at 5000 rpm. The clear supernatant was filtered through a disposable syringe filter and injected. This solution contained about 50 – 60 µg mL^{-1} MFP. The chromatogram is shown in Figure 2. The results obtained were compared to those given by the comparison method for MFP analysis [15].

2.2.2. Determination of free phosphate in some pharmaceutical materials

Phosphate was determined in betamethasone, dexamethasone and prednisolone sodium phosphate salts. To carryout the analysis; 0.25 g of powdered raw material was accurately weighed into a 100-mL volumetric flask and dissolved completely in water, exact amount of the reference phosphate material was added then the volume was completed to the mark. The solution was filtered through a disposable syringe filter. The experiment was carried out in triplicate and each solution was injected in triplicate. The chromatograms are shown in Figures 3 (a – c). The results obtained were compared to those given by the comparison method for phosphate analysis [16].

2.2.3. Determination of iodide in amiodarone hydrochloride pharmaceutical material

To carry out the analysis; 0.30 g of powdered amiodarone hydrochloride raw material was accurately weighed, exact amount of the standard iodide material was added. The solution was transferred into a beaker containing 60 mL water and heated to 80 ^{o}C. After complete dissolution, it was cooled and the solution was quantitatively transferred into a 100-mL volumetric flask. It was sonicated for five minutes and the volume was completed to the mark with water then filtered through a disposable syringe filter. The experiment was carried out in triplicate and each solution was injected in triplicate. The chromatogram is shown in Figure 4. The results obtained were compared to those given by the comparison method for iodide analysis [17].

3. Results and Discussion

Reversed phase columns are permanently coated with large hydrophobic quaternary amines to give a charged surface that can be used for ion exchange. These columns are superior to dynamic ion exchangers (ion pair chromatography) for indirect detection because the presence of the dynamic modifier in the mobile phase can complicate detector response [18]. Indirect UV detection means that a UV absorbing ion is included in the mobile phase and distributed to the solid phase. Injected solutes will influence the distribution of the detectable ion by binding or displacement and give rise to a detector response, even when the solute lacks detectable properties [19]. For indirect UV detection, the absorbance of the mobile phase is monitored. The changes in its concentration as the sample anion is eluted can be related to the

concentration of that anion in the eluate. The basic principle of the detection mechanism is exchange of visualization-reagent anion for sorbed sample anion.

In this study, all conditions affecting chromatographic performance were carefully studied. The most appropriate mobile phase composition was found to be 1.0 mM sodium salicylate – 5 % methanol except for phosphate where 0.5 mM sodium salicylate – 10 % methanol was used as mobile phase. Flow rate of 2.0 mL $min.^{-1}$ with indirect detection at 290 nm were used. The choice was based on the best peak shape, resolution and the lowest peak tailing. Under the stated conditions, the system peak was positive at retention time of ca. 0.7 minutes which was due to the displacement of salicylate ions from the charged column surface by the injected sample. The anions were eluted later as negative peaks (see Figures 1 (a – c)).

Under the chromatographic conditions stated, phosphate eluted as a negative peak at ca. 2.5 minutes. These conditions were suitable when analyzing pure phosphate and the system peak was well separated from phosphate peak. On the other hand, the presence of the raw material caused a great increase in the system peak. This increase was due to displacement of more salicylate anion. Consequently, the system peak became very close to the phosphate peak. This decrease in peak resolution made the calculation of peak area somewhat difficult and irreproducible. The use of 0.5 mM sodium salicylate solution-10% methanol as mobile phase instead of that mentioned before showed increased retention of phosphate (retention time ca. 4.6 minutes). So, phosphate could be determined accurately and precisely in pharmaceutical raw materials. The chromatographic performance data for analysis of some inorganic anions by the proposed method are summarized in Table 1.

Table 1. The chromatographic performance data for analysis of some inorganic anions by the proposed method.

Parameters	MFP	Phosphate	Iodide
Retention time (min.)	4.12 ± 0.12	2.54 ± 0.06	3.25 ± 0.11
Peak width at 10 % height	0.61	0.53	0.47
Tailing factor	0.92	1.47	0.98
Number of theoretical plates	716	365	751
Regression equation	y = 11049 x - 565.8	y = 7596 x + 5841.5	y = 8311 x - 17936
Correlation coefficient	0.9998	1.0000	0.9999
Mean ± SD	100.44 ± 1.45	99.95 ± 0.36	100.42 ± 2.23
Working range (µg mL^{-1})	10.0 – 100.0	10.0 – 120.0	10.0 – 120.0
LOD (µg mL^{-1})	4.0	4.0	4.0
LOQ (µg mL^{-1})	10.0	10.0	10.0

3.1. Optimization of the chromatographic performances.

3.1.1. Sodium salicylate concentration.

The influence of sodium salicylate concentration on retention time of the analytes is illustrated in Figures. 5 (a and b) which show the chromatograms obtained for phosphate using 0.25 mM and 1.00 mM aqueous sodium salicylate as mobile phase, respectively. Sodium salicylate (pH 6.4) in a concentration range of 0.1 – 1.5 mM were tried as the mobile phase at a flow rate of 2.0 mL min^{-1}. In these trials the retention time decreased with increased salicylate concentration. Higher concentrations of salicylate (more than 1.5 mM) caused saturation of detector at the wavelength used for detection. On the other hand, decreasing the salicylate concentration resulted in higher retention times and consequently appearance of more broad peaks which made calculation of peak area somewhat difficult and irreproducible.

3.1.2. Effect of solvent modifier.

The results obtained with a 1.0 mM sodium salicylate solution – methanol (95:5) at 2.0 mL min^{-1} flow rate showed a good separation for the inorganic anions studied. When aqueous sodium salicylate solution was used, the peaks are eluted early and bad resolution between the system and sample anion peaks was observed. Higher percent of methanol resulted in increased retention time and broad peaks which is because the addition of organic solvent change ion activity. Concentrations of methanol higher than 10 % (v/v) were not tried because dissolution of *n*-cetylpyridinium ions adsorbed in the stationary phase could occur which, in turn, decreases the lifetime of the column [12].

3.1.3. Choice of detection wavelength.

Sodium salicylate spectrum showed two maxima, at 230 and 290 nm. Measurements are made at 290 nm, at which sodium salicylate has lower absorbitivity, to avoid higher response error and saturation of detector [20].

3.2. Method validation.

3.2.1. Accuracy and precision.

Tables 2 (a – c) represent the results for the validation studies of the proposed technique. The accuracy as percent relative error (% Er) was found to be within 0.85 – 0.94 % for the intra-day assay (n = 3) and within 0.34 – 0.72 % for the interday assay (n = 3) which prove a satisfactory accuracy. The precision as percent relative standard deviation (% RSD) is represented. The repeatability of the assays was found to be within 1.47 – 1.63 % (n = 3) and

the reproducibility of the assays was found to be within 0.58 – 1.25 % (n = 3) which were within the accepted limit for % RSD.

Table 2. Intra-day and interday data for some inorganic anions by the proposed chromatographic method.

(a) MFP.

Parameters	Intra-day	Inter-day
% Recovery	102.73 99.85 99.94	99.04 100.16 99.88
Mean ± S.D.	101.01 ± 1.64	99.69 ± 0.58
% R.S.D.	1.62	0.58

(b) Phosphate.

Parameters	Intra-day	Inter-day
% Recovery	99.03 100.21 101.97	100.40 99.45 98.97
Mean ± S.D.	100.40 ± 1.48	99.61 ± 0.73
% R.S.D.	1.47	0.73

(c) Iodide.

Parameters	Intra-day	Inter-day
% Recovery	101.90 99.68 98.72	100.01 99.51 101.90
Mean ± S.D.	100.10 ± 1.63	100.47 ± 1.26
% R.S.D.	1.63	1.25

N.B. Each result is the average of three separate determinations.

3.2.2. Concentration ranges and calibration graphs.

Under the above described experimental conditions, linear relationships were established by plotting concentrations against their absolute peak areas for each compound. Peak areas are used for quantitation, because indirect detection is an inherently nonlinear system, and peak hieghts, which are more sensitive to peak shape, usually gave calibration

plots that had much smaller linear ranges [18]. The regression equations, regression coefficients and linear ranges are listed in Table 1. Statistical analysis of the results obtained for pure MFP, phosphate and iodide by both the proposed and the comparison methods [15 – 17] revealed no significant difference between the performance of the two methods as indicated by the F-test and student t-test (Tables 3 (a – c)).

Table 3. Statistical analysis of the results obtained for analysis of some inorganic anions in pure form by the proposed chromatographic method and reference methods.

(a) MFP analysis.

Parameter	Proposed method [a]	Comparison method [15]
Mean [c] ± S.D.	100.44 ± 1.45	100.03 ± 2.45
% R.S.D.	1.44	2.45
F	2.83	(4.88) [d]
t	0.36	(2.17) [d]

(b) Phosphate analysis.

Parameter	Proposed method [b]	Comparison method [16]
Mean [c] ± S.D.	100.57 ± 2.50	99.44 ± 2.75
% R.S.D.	2.49	2.77
F	1.21	(5.41) [d]
t	0.68	(2.30) [d]

(c) Iodide analysis.

Parameter	Proposed method [a]	Comparison method [17]
Mean [c] ± S.D.	100.42 ± 2.23	99.79 ± 1.37
% R.S.D.	2.22	1.37
F	2.64	(9.01) [d]
t	0.50	(2.30) [d]

a Chromatographic conditions as under Experimental section.
b Under the modified conditions.
c Average of at least three different determinations.
d Values in parenthesis are theoretical values at (P = 0.05).

3.2.3. Limit of quantitation (LOQ) and limit of detection (LOD).

The limit of quantitation (LOQ) was determined by establishing the lowest concentration that could be measured with acceptable accuracy and precision at S/N = 10. Under the proposed chromatographic conditions, MFP, phosphate and iodide could be quantified at concentration of 10.0 µg mL^{-1}. The limit of detection (LOD) was determined by establishing the minimum level at which the analyte could be reliably detected (S/N = 3) and it was found to be 4.0 µg mL^{-1} for MFP, phosphate and iodide.

3.3. Applications

3.3.1. Determination of MFP in toothpastes

The proposed method was successfully applied to the assay of MFP in toothpastes. The average percent recovery for MFP in commercially available toothpaste was $0.7755 \pm 8.80 \times 10^{-3}$ (Tables 4 and 5).

Table 4. Analysis of MFP in toothpastes by the proposed chromatographic method and comparison method [15].

Sample	Proposed method (% Recovery)	Comparison method [15] (% Recovery)
Sample (1.102 g % MFP)	0.7843 0.7755 0.7667	0.8042 0.8459 0.7951
Mean ± S.D.	$0.7755 \pm 8.80 \times 10^{-3}$	$0.8151 \pm 27.08 \times 10^{-3}$

Table 5. Statistical analysis of the results obtained for analysis of MFP in toothpastes by the proposed chromatographic method and comparison method [15].

Parameter	Proposed method [a]	Reference method [15]
Mean [b] ± S.D.	$0.7755 \pm 8.80 \times 10^{-3}$	$0.8151 \pm 27.08 \times 10^{-3}$
% R.S.D.	1.13	3.32
F	14.00	(19.00) [c]
t	2.50	(2.77) [c]

a Chromatographic conditions as under Experimental section.
b Average of at least three different determinations.
c Values in parenthesis are theoretical values at (P = 0.05).

3.3.2. Determination of phosphate in pharmaceutical materials

The proposed method was successfully applied to the assay of phosphate in spiked samples of pharmaceutical materials. The average percent recoveries for phosphate in betamethasone sodium phosphate, dexamethasone sodium phosphate and prednisolone sodium phosphate were 0.344 ± 0.0227, 0.255 ± 0.0285 and 1.053 ± 0.0820, respectively (Tables 6 and 7).

Table 6. Analysis of phosphate in pharmaceutical materials under the modified chromatographic conditions and comparison method [16]

Sample	Proposed method (% Recovery)	Comparison method [16] (% Recovery)
Betamethasone sodium phosphate	0.368 0.323 0.341	0.300 0.365 0.319
Mean ± S.D.	0.344 ± 0.0227	0.328 ± 0.0334
Dexamethasone sodium phosphate	0.256 0.226 0.283	0.258 0.218 0.248
Mean ± S.D.	0.255 ± 0.0285	0.242 ± 0.0209
Prednisolone sodium phosphate	0.959 1.090 1.110	1.111 0.952 1.044
Mean ± S.D.	1.053 ± 0.0820	1.036 ± 0.0796

Table 7. Statistical analysis of the results obtained for analysis of phosphate in pharmaceutical materials by the proposed chromatographic method and comparison method [16]

(a) Betamethasone sodium phosphate

Parameter	Proposed method [a]	Comparison method [16]
Mean [b] ± S.D.	0.344 ± 0.0227	0.328 ± 0.0334
% R.S.D.	6.60	10.18
F	2.15	(19.00) [c]
t	0.69	(2.77) [c]

(b) Dexamethasone sodium phosphate

Parameter	Proposed method [a]	Comparison method [16]
Mean [b] ± S.D.	0.255 ± 0.0285	0.242 ± 0.0209
% R.S.D.	11.18	8.64
F	1.84	(19.00) [c]
t	0.64	(2.77) [c]

(Table 7 continued)

(c) Prednisolone sodium phosphate

Parameter	Proposed method [a]	Comparison method [16]
Mean [b] ± S.D.	1.053 ± 0.0820	1.036 ± 0.0796
% R.S.D.	7.79	7.68
F	1.06	(19.00) [c]
t	0.26	(2.77) [c]

a Under the modified conditions.
b Average of at least three different determinations.
c Values in parenthesis are theoretical values at (P = 0.05).

3.3.3. Determination of iodide in amiodarone hydrochloride pharmaceutical material

The proposed method was successfully applied to the assay of iodide in spiked samples of amiodarone hydrochloride. The average percent recovery for iodide was $10.312 \times 10^{-3} \pm 0.336 \times 10^{-3}$ (Table 8 and 9).

Table 8. Analysis of iodide in amiodarone hydrochloride by the proposed chromatographic method and comparison method [17]

Sample	Proposed method (% Recovery)	Comparison method [17] (% Recovery)
Amiodarone hydrochloride	10.667×10^{-3} 10.000×10^{-3} 10.270×10^{-3}	9.870×10^{-3} 10.030×10^{-3} 10.270×10^{-3}
Mean ± S.D.	$10.312 \times 10^{-3} \pm 0.336 \times 10^{-3}$	$10.057 \times 10^{-3} \pm 0.200 \times 10^{-3}$

Table 9. Statistical analysis of the results obtained for analysis of iodide in amiodarone hydrochloride by the proposed chromatographic method and comparison method[17]

Parameter	Proposed method [a]	Comparison method [17]
Mean [b] ± S.D.	$10.312 \times 10^{-3} \pm 0.336 \times 10^{-3}$	$10.057 \times 10^{-3} \pm 0.200 \times 10^{-3}$
% R.S.D.	3.26	1.99
F	2.75	(19.00) [c]
t	1.29	(2.77) [c]

a Chromatographic conditions as under Experimental section.
b Average of at least three different determinations.
c Values in parenthesis are theoretical values at (P = 0.05).

4. Conclusion

The results indicate that the ion-interaction LC assay demonstrates linearity, precision, accuracy at concentrations ranging from 10.0 to 120.0 µg mL^{-1}. Easy handling and preparation of the coated column, no need for any special pretreatment of the sample and satisfactory reproducibility of the results suggest that this method may be implemented to routine laboratory analysis. According to the needs, the coated column can be conveniently regenerated for other analytical purposes which serves the advantage of economy of material.

List of non-standard abbreviations:

MFP : sodium monofluorophosphate
ODS : octadecyl silane
pK : - log the ionization constant

References

1. Ito K, Ariyoshi Y, Tanabiki F and Sunahara H (1991) Anion chromatography using octadecylsilane reversed-phase columns coated with hexadecyltrimethylammonium and its application to nitrite and nitrate in sea-water. Anal. Chem. 63(3): 273.
2. Michigami Y and Yamamoto Y (1992) Behaviour of the system peak in ion chromatography with indirect ultra-violet absorption detection and trimellitate as eluent. J. Chromatogr. A. 623(1): 148.
3. Jun X, Lima J L F C and Montenegro M C B S M (1997) A study of a permanently coated polymeric column for simultaneous separation of inorganic anions and monocarboxylic acids. Anal. Chim. Acta. 339(3): 231.
4. Krokhin O V , Svintsova N V , Obrezkov O N and Shpigun O A (1997) Modified silica gel for the determination of anions by single-column ion chromatography. J. Anal. Chem. (Transl. of Zh. Anal. Khim). 52(11): 1066.
5. Ito K, Shoto E and Sunahara H (1991) Ion chromatography of inorganic iodine species using C_{18} reversed-phase columns coated with cetyltrimethylammonium. J. Chromatogr. A. 549: 265.
6. Ito K, Ariyoshi Y and Sunahara H (1992) Determination of inorganic anions in salt solutions by ion chromatography using C_{18} reversed-phase columns coated with cetyltrimethylammonium. J. Chromatogr. A. 598 (2): 237.
7. Ohta K and Tanaka K (1995) Retention behaviour of inorganic anions and organic acids on a polymer-coated C18 column coated with cetyltrimethylammonium ion by ion chromatography with indirect UV spectrophotometric detection. Bunseki Kagaku 44(9): 713.

8. Ito K (1997) Separation behaviour of inorganic anions on an octadecyl-bonded silica column coated with cetyltrimethylammonium by phthalate and salicylate eluents. J. Chromatogr. A. 764(2): 346.
9. Nagashima H and Okamoto T (1999) Determination of inorganic anions by ion chromatography using a graphitized carbon column dynamically coated with cetyltrimethylammonium ions. J. Chromatogr. A. 855 (1): 261.
10. Yoshikawa K, Okamura M, Inokuchi M and Sakuragawa A (2007) Short communication. Ion chromatographic determination of organic acids in food samples using a permanent coating graphite carbon column. Talanta 72 (1): 305.
11. Lei Z, Tian S and Cheng J (1989) Column coated with hexadecylpyridinium for separation of inorganic anions by ion-interaction [reversed-phase] chromatography. Sepu 7(1): 44.
12. Jun X, Lima J L F C and Montenegro M C B S M (1996) Simultaneous determination of inorganic anions and carboxylic acids in wine using isocratic separation on a permanently coated reversed-phase column and UV indirect detection. Anal. Chim. Acta. 321 (2-3): 263.
13. Michigami Y, Morooka M and Ueda K (1996) Short communication. Determination of sulphite and sulphate by ion chromatography using a weakly basic phthalate eluent. J. Chromatogr. A. 732 (2): 403.
14. Li J, Zhu Y and Guo Y (2006) 18th Annual International Ion Chromatography Symposium. Fast determination of anions on a short coated column. J. Chromatogr. A. 1118 (1): 46.
15. Pavic M, Carevic D and Cimerman Z (1999) Potentiometric determination of monofluorophosphate in dentifrice: a critical discussion and a proposal for new improved procedures. J. Pharm. Biomed. Anal. 20(3): 565.
16. Tsang S, Phu F, Baum M M and Poskrebyshev G A (2007) Determination of phosphate/arsenate by a modified molybdenum blue method and reduction of arsenate by $S_2O_4^{2-}$. Talanta 71 (4): 1560.
17. Koh T and Ono M (1988) Determination of iodide by ion-pair method with methylene blue. Analyst 113: 945.
18. Cassidy R M and Elchuk S (1985) Fixed-site ion exchanger for liquid chromatographic determination of multifunctional carboxylic acids Anal. Chem. 57: 615.
19. Hackzell L, Rydberg T and Schill G (1983) Construction of systems for detection and quantitation by UV-absorbing mobile-phase ions in reversed-phase chromatography J. Chromatogr. A. 282: 179.
20. Gao J, Yang W, Kang J, Hou J and Bian H (1997) Anion separation by high-performance ion chromatography coupled with indirect photometric detection and 1,2-

dihydroxybenzene-3,5-disulfonate or sodium sulfosalicylate as the eluent J. Chromatogr. A. 789(1-2): 195.

Received: 13 March 2008 ; Accepted: 04 April 2008

Corresponding author: Mohamed Salem Rizk
Address: P.O.Box.College of Pharmacy Helwan University, Ain Helwan – Cairo (POB 11795) Egypt

Phone: (002) 02 23692597

Fax: (002) 02 25541601

Email: aliaa.shallan@yahoo.com