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Journal of Chromatography A, 670 (1994) 223–228

JOURNAL OF CHROMATOGRAPHY A

Determination of fluoride in feed mixtures by capillary isotachophoresis

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(First received August 19th, 1993; revised manuscript received January 24th, 1994)

Abstract

An isotachophoretic (ITP) method for the determination of fluoride in feed mixtures was developed. A sample of feed mixture, after extraction with 1 *M* HCl, was analysed using a ZKI 02 column-coupling isotachopherograph. Leading electrolytes for presentation and analytical capillaries consisted of 0.008 *M* HCl–0.022 *M* ε-aminocaproic acid (EACA)–0.001 *M* $CaCl_2$–0.05% hydroxypropylmethyl cellulose (HPMC) and 0.002 *M* HCl–0.005 *M* EACA–0.05% HPMC, respectively. The terminating electrolyte was 0.01 *M* tartaric acid. The fluoride released from samples by microdiffusion in 25% perchloric acid was determined using an Ionosep 900.1 single capillary isotachopherograph with 0.002 *M* HCl–0.005 *M* EACA–0.05% HPMC as the leading electrolyte and 0.002 *M* tartaric acid as the terminating electrolyte. The detection limit, depending on the sample treatment, was as low as 4 μg/g as fluoride. A comparison of the developed ITP method with ion-selective electrode method was carried out.

1. Introduction

Fluoride is included in the category of harmful substances and its content in feeds is limited (150 ppm in the Czech Republic [1]). Fluoride levels in feeds should not exceed the authorised limits, and therefore it is necessary to have a suitable method available for its determination. Spectrophotometric determination with xylenol orange can be used [2], where an ashed sample is analysed after isolation of fluoride by steam distillation from perchloric acid. Another method involves microdiffusion from perchloric acid and spectrophotometric determination with lanthanum alizarin complexone [2]. Tušl [3] developed a method for the determination of fluoride in phosphates with a fluoride ion-selective electrode. Torma [4] used a fluoride ion-selective electrode to determine fluoride released from an animal feed by extraction with 1 *M* hydrochloric acid. He compared this method with the official AOAC method (ashing the sample followed by steam distillation and titration) and reported good agreement of the results. Singer and Ophaug [5] determined fluoride in some foods with a fluoride ion-selective electrode. Fluoride was isolated from ashed and unashed sample by heat- or silicone-facilitated diffusion. Higher levels of fluorine were found in ashed samples (total fluorine) than in unashed samples (ionic acid-labile fluorine).

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0021-9673/94/$07.00
SSDI 0021-9673(94)00076-L

2. Experimental

2.1. Chemicals

Analytical-reagent grade chemicals were used unless indicated otherwise.

Hydrochloric acid (Normanal), 0.1 mol/l, acetic acid (99%), hydrochloric acid (35–37%), and perchloric acid (70%), were obtained from Lachema (Brno, Czech Republic), β-alanine (BALA) (99%+), and ε-aminocaproic acid (EACA), from Janssen Chimica (Beerse, Belgium), hydroxypropylmethylcellulose (HPMC) 4000 from Aldrich (Milwaukee, WI, USA), and tartaric acid, disodium phosphate, sodium hydroxide and calcium chloride from Lachema.

2.2. Samples

Animal feed mixtures were as follows: sample A = feed mixture for pigs of up to 35 kg of live mass; sample B = feed mixture for broilers; sample C = feed mixture for calves; and sample D = feed mixture prepared by mixing of phosphate B (see below) with sample A.

The phosphate samples were (A) dicalcium phosphate exported from The Netherlands and (B) dicalcium phosphate purchased from Fosfa Poštorná (Czech Republic).

2.3. Instrumentation

A ZKI 02 column-coupling isotachopherograph; a PTFE preseparation capillary (170 × 0.8 mm I.D.) and a PTFE analytical capillary (170 × 0.3 mm I.D.) were obtained from Villa (Slovak Republic). An Ionosep 900.1 volume-coupling single-capillary isotachopherograph (preseparation part 50 × 1 mm I.D., separation part 150 × 0.45 mm I.D. and detection part 70 × 0.40 mm I.D.) was obtained from Recman-Laboratorní Technika (Czech Republic).

A TZ 4200 chart recorder (Laboratorní přístroje, Prague, Czech Republic), a QP-205/1 pH meter (Radelkis, Budapest, Hungary), a Crytur fluoride ion-selective electrode (Monokrystaly Turnov, Czech Republic) and a Model AT/286 IBM-compatible microcomputer were used.

2.4. Conditions of analysis

The electrolyte systems and running parameters are presented in Table 1.

2.5. Calibration

An external standard calibration method was used with sodium fluoride as the standard. For the determination of fluoride on ZKI 02, four

Table 1
Electrolyte systems and running parameters

Electrolyte system[a]	Capillary	Current (μA)
(I) LE_1: 8 m*M* HCl–22 m*M* EACA–1 m*M* $CaCl_2$–0.05% HPMC, pH 4.45	Preseparation	Initial 300 Detection 250
LE_2: 2 m*M* HCl–5 m*M* EACA–0.05% HPMC, pH 4.25	Analytical	Initial 30 Detection 5
TE: 10 m*M* tartaric acid (for determination of fluoride in phosphates, 10 m*M* sodium hydrogenphosphate)		
(II) LE: 2 m*M* HCl–5 m*M* EACA–0.05% HPMC		Initial 40 Detection 5
TE: 2 m*M* tartaric acid		

[a] I = Electrolyte system for the determination of fluoride released by extraction with 1 *M* HCl using the ZKI 02 (analysis time 22–27 min depending on sample dilution). II = Electrolyte system for determination of fluoride released by heat-facilitated diffusion from 25% perchloric acid on the Ionosep 900.1 (analysis time 10 min). LE = Leading electrolyte; TE = terminating electrolyte.

concentration levels were measured (0.2–1 μg/ml). The standard solution was injected into the isotachopherograph by a sample valve (40 ml) and by a 100-ml Hamilton syringe. For the determination of fluoride on the Ionosep 900.1, four calibration points were measured (0.2–1 μg/ml). Standard solutions were injected by a sample valve (20 μl).

2.6. Sample preparation

Determination of fluoride released by extraction of 1 M HCl on the ZKI 02

Into a 200-ml volumetric flask, 2–5 g of an animal feed mixture were weighed and extracted with 20 ml of 1 *M* HCl with magnetic stirring for 30 min at ambient temperature. The volume was then made up to 200 ml with distilled water. After filtration and dilution (tenfold with deionized water), the solution obtained was injected into the isotachopherograph. For the determination of fluoride in dicalcium phosphates, 500 mg of sample were weighed into a 100-ml volumetric flask and extracted with 20 ml of 1 *M* HCl with magnetic stirring under the above-mentioned conditions. The volume was made up to 100 ml with distilled water. After dilution (20–50-fold with deionized water), the solution obtained was analysed using the ZKI 02 isotachopherograph.

Determination of fluoride released by heat-facilitated diffusion from 25% perchloric acid on the Ionosep 900.1

On the bottom of a polyethylene Petri dish, 1–2 g of feed mixture or 150 mg of dicalcium phosphate were placed and 25% perchloric acid (8 ml) was added. The diffused fluoride was trapped in 1 ml of 0.5 *M* NaOH that was placed in a smaller dish at the bottom of the Petri dish. The rims of both parts of the Petri dishes were coated with silicone grease. The dishes were placed in a thermostat at 60°C for 16 h. After heat-facilitated diffusion, the contents of the smaller dish were transferred into a 25-ml volumetric flask (stock solution). A 1–2 ml volume of the stock solution was pipetted into a 10-ml volumetric flask, 1 ml of 0.01 *M* acetic acid was added and the volume was made up with deionized water. The solution was then analysed using the Ionosep 900.1 isotachopherograph.

3. Results and discussion

For the determination of fluoride released by the extraction with 1 *M* HCl, an isotachopherograph with a column-coupling system (ZKI 02) is necessary because of the high concentration of chloride. In addition, various feed samples can have different qualitative compositions, causing difficulties in the selection of an electrolyte system for the determination of fluoride released by extraction with HCl. First leading electrolytes without co-counter ions were tested. These electrolytes were chosen with the support of computer steady-state simulation in order to prevent mixed zones of fluoride with anions, which are present in feed mixtures (oxalate, tartrate, formate, pyrophosphate).

Leading electrolytes with BALA, EACA and histidine counter ions were tested. As the sample contains a very low concentration of fluoride it is necessary to apply a driving current that is as low as possible (5 μA), allowing quantitative analysis. Therefore, it was also necessary to use a leading electrolyte with a lower concentration of the leading anion to ensure sufficiently sharp boundaries between the zones. We tested leading electrolytes with concentrations 2 m*M* of leading anion (HCl) and 5 m*M* of counter ion (the same counter ion as in the preseparation capillary). However, it was found that fluoride created a mixed zone with unknown anions in all these systems. Therefore, these leading electrolytes were optimized through the addition of a co-counter ion (Ca) to the leading electrolyte in the preseparation capillary. The best results were obtained with the system presented in Table 1. In this system the separation of fluoride from interfering ionic compounds due to a complex-forming equilibrium with Ca was achieved.

For the determination of fluoride in feed mixtures, 10 m*M* tartaric acid served as the terminating electrolyte. The choice of this terminator is very important, because some anions

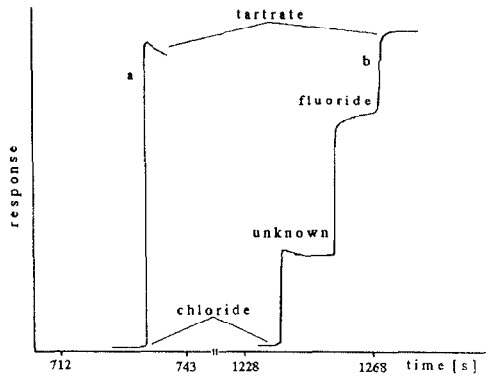

Fig. 1. Isotachopherogram of animal feed mixture (sample C), measured with the ZKI 02 column-coupling isotachopherograph: (a) preseparation capillary; (b) analytical capillary. The isotachopherograms were measured with a contact conductivity detector. It is clear from the analytical conditions (see Table 1) that a 1 mm step length of fluoride in the preseparation capillary gives a 50 mm step length in the analytical capillary. The optimum timing of the column switching was found to be 3 s before the fluoride zone reached the bifurcation block.

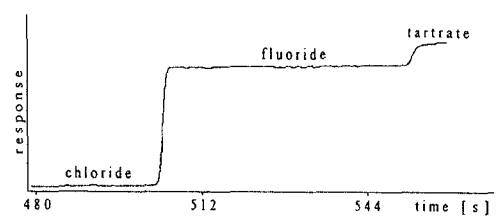

Fig. 2. Isotachopherogram of a model mixture of the 2 mg/ml of fluoride acquired with the Ionosep 900.1 single-capillary isotachopherograph. As microdiffusion represents purification process, the isotachopherogram of the real sample analysis also contains only one step, fluoride. For details, see text (Table 1).

that form complexes with Ca have an effective mobility lower than that of the terminator. Hence these anions remain the preseparation capillary and do not load the very low separation capacity in the analytical capillary.

For the determination of fluoride released by heat-facilitated diffusion from 25% perchloric acid, a single-capillary isotachopherograph can be used. In this instance the selection of the electrolyte system is easier, because only fluoride and some volatile acids are trapped in the solution of NaOH. With regard to the concentration of fluoride in the alkali solution (see Section 2.6), the same leading electrolyte as for the analytical capillary of the ZKI 02 analyser was chosen (see Table 1). Although the dilution of the leading electrolytes also decreases its separation capacity, it was confirmed by the standard addition technique that the fluoride from the sample is separated and determined correctly using the above-mentioned leading electrolyte. The terminating electrolyte was 2 m*M* tartaric acid. Although the step height of fluoride is close to that of the tartrate, we verified that fluoride migrates correctly even at a concentration exceeding our calibration range twofold. Calibration results are given in Table 2.

The ITP method developed for the determination of fluoride was tested on a series of phosphate samples and animal feed mixtures. The results obtained are summarized in Table 3. An isotachopherogram of animal feed mixture (sample C) analysed on the ZKI 02 column-coupling isotachopherograph is shown in Fig. 1. Fig. 2 shows an isotachopherogram of a model mix-

Table 2
Results of calibration analyses

Isotachopherograph	RSH[a]	Calibration equation	R_{xy}[b]	Sample loading
ZKI 02	0.74	$y = 51.2x - 0.5$[c]	0.999	Via sample valve
ZKI 02	0.74	$y = 1.37x + 1.27$[d]	0.999	Via syringe
Ionosep 900.1	0.81	$y = 603.2x - 5.3$[e]	0.999	Via sample valve

[a] RSH = Relative step height.
[b] R_{xy} = Correlation coefficient.
[c] y = Step length in mm; x = concentration of fluoride in μg/ml; chart speed 6 cm/min.
[d] y = Step length in mm; x = amount of fluoride in ng; chart speed 6 cm/min.
[e] y = Step length in sample; x = concentration of fluoride in μg/ml; sample rate 20/s.

Table 3
Results of fluoride determination (extraction with 1 *M* HCl, analysis on ZKI 02 column-coupling isotachopherograph and heat-facilitated diffusion from 25% $HClO_4$ with analysis on Ionosep 900.1 single-capillary isotachopherograph) in phosphate and animal feed mixture samples

Sample	Fluoride content (mg/kg)	
	ZKI 02[a]	Ionosep[b]
Animal feed A	16	10
Animal feed B	8	8
Animal feed C	53	45
Animal feed D	171	117
Phosphate A	920	890
Phosphate B	1510	1740

For detailed conditions of analysis, see text.
[a] Average of three replicate analyses; R.S.D. = 1.9% ($n = 10$, sample C).
[b] Average of two replicate analyses; R.S.D. = 4% ($n = 10$, sample C).

ture (2 μg of fluoride/ml) analysed on IONOSEP 900.1.

The technique based on extraction of a sample with hydrochloric acid gives recoveries between 80 and 100% (determined with the ZKI 02 analyser) and the method based on heat-facilitated diffusion from perchloric acid gives recoveries between 60 and 120% (determined with the Ionosep analyser). The poorer recoveries and higher R.S.D. values (see Table 3) obtained with the latter technique are probably due to the low sample mass (1–2 g) with respect to heterogeneity of feed mixtures. This disadvantage could be eliminated by the use of a device for heat-facilitated diffusion, enabling larger amounts of sample to be used. The different contents of fluoride found by the two isotachophoretic methods (171 ppm with the ZKI 02 and 117 ppm with the Ionosep 900.1) are probably caused by imperfect homogenization of sample. Sample D was prepared in our laboratory by addition of phosphate sample B to animal feed sample A obtain a sample with a higher fluoride content (150 ppm). The heat-facilitated diffusion from perchloric acid seems to be a more advantageous technique than the extraction method, especially for the determination of fluoride in phosphate samples. The sample amount of dicalcium phosphate (150 mg) ensures perfect solubility under the conditions of microdiffusion and provides a sufficient step length of fluoride on the isotachopherogram. For example, the sample phosphate B was diluted 50-fold after extraction in 1 *M* HCl and the step length of fluoride on the isotachopherogram was therefore only 5 s (ZKI 02) whereas the step length of fluoride for this sample treated by the heat-facilitated diffusion method was over 30 s (Ionosep 900.1).

Analyses of ashed samples were carried out. Fluoride was released from the ashed sample by heat-facilitated diffusion. It was found that during the ashing of a sample part of the fluoride escaped (for detailed conditions of ashing, see ref. 5), because the ashed sample contained less fluoride that the unashed sample. The escape of fluoride could be suppressed by the addition of NaOH or Na_2CO_3 before ashing, but we did not try this. It was also found that it is not possible to dissolve the ashed sample by the classical method [6], because all the fluoride escaped as hydrogen fluoride owing to the action of concentrated hydrochloric acid and therefore no fluoride was found in the sample. Partial fluoride escape was also observed during the extraction by a sample with 1 *M* HCl. Therefore, distilled water, 1 *M* phosphoric acid and more dilute hydrochloric acid (0.01, 0.1 and 0.5 *M*) were tested as extractants. We found that if the content of fluoride in the feed mixture does not exceed 20 ppm (determined after extraction with 1 *M* HCl), all of these solvents are suitable. When the content of fluoride exceeds 20 ppm, extraction with 1 *M* HCl gave the best results, probably owing to better solubilization of phosphates containing fluoride.

Comparative analyses using an ion-selective electrode (ISE) were carried out. On the basis of the results obtained it is concluded that the ITP method is more suitable than the ISE method for the determination of fluoride in feed mixtures and/or in phosphate samples. The ISE method gave recoveries of up to 180%, *i.e.*, only semi-quantitative results for fluoride content. The only advantage of the ISE method is the rela-

tively short analysis time. However, the rate of voltage stabilization between the ISE and the reference electrode is dependent on the condition of the ISE. The experiments described lasted 6 months. At the beginning of this period the voltage stabilization took 2 min and this time had increased to 10 min by the end of experimental work. A daily decrease of the ISE sensitivity was observed and a calibration analysis had to be carried out every day. Although the ISE method is highly recommended in some papers for the determination of fluoride in feed mixtures, our experience showed that the use of this technique is questionable. In addition, a large consumption of chemicals, *i.e.*, 16 g of sodium citrate and *ca.* 8 g of sodium acetate per analysis, is necessary. In contrast, the ITP calibration graph did not change with time. Nevertheless, we recommend that the calibration is verified every time a new leading electrolyte is prepared.

The detection limits for feed mixtures analysis under the experimental conditions adopted using the ZKI-02 and Ionosep isotachopherographs were 6 and 4 mg/kg as fluoride, respectively, and those for fluoride determination in phosphate samples were 120 and 50 mg/kg, respectively. These values are much lower than the admissible fluoride content in phosphate samples (2000 mg/kg) or in feed mixtures (150 mg/kg).

On the basis of the results obtained and the fact that there are few methods for fluoride determination, we recommend ITP as suitable technique for this purpose. With advantages such as the relatively simple sample preparation and very low consumption of chemicals per analysis, resulting in low running costs, the ITP technique presented could find practical use in this field.

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