Determination of trace amounts of fluoride in raw materials for pharmaceuticals by gas–liquid chromatography

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ABSTRACT

Trace amounts of inorganic fluoride present in raw materials for pharmaceuticals are converted into an organic compound by trimethylchlorosilane at acidic pH. The trimethylfluorosilane formed is determined by gas–liquid chromatography with flame ionization detection using isopentane as an internal standard. Seventeen pharmaceutical raw materials were analysed by this method. The fluoride levels found varied from 0.10 ppm in potassium chloride to 162 ppm in a sample of tribasic calcium phosphate. Coefficents of variation were 0.6–0.8% and the sensitivity was 0.01 ppm. This method is suitable for the determination of trace amounts of fluoride in raw materials for pharmaceuticals because of its simplicity, its accuracy and sensitivity.

INTRODUCTION

The presence of fluoride impurities in some inorganic raw materials for pharmaceuticals such as alkaline earth salts is well known. These impurities result from contamination by minerals such as fluorapatite $[Ca_{10}F_2(PO_4)_6]$ or francolite $[Ca_{10}F_2(PO_4)_6, xCaCO_3]$ found in the ores of these raw materials.

Fluoride is considered as an essential trace element necessary for bone formation and the prevention of dental caries. However, an excess of fluoride is toxic and can induce diverse bone diseases such as fluorosis, osteoporosis and skeletal fragility [1–4]. The cytotoxicity of fluoride has also been reported [5–8]. For these reasons, different pharmacopoeia give a maximum concentration of fluoride in certain raw materials. Two methods of determination are

used in the main pharmacopoeia. A spectrophotometric method after a distillation procedure is described in the European [9], French [10] and British Pharmacopoeia [11]. This technique is tedious, time-consuming and insensitive. A potentiometric method with a specific fluoride electrode is described in the United States Pharmacopoeia (USP) [12]. However, this technique is not linear at less than 50 μg/l fluoride and can give errors as a result of interference from OH$^-$ [13]. The lengthy equilibration period of the electrodes at low concentrations is also inconvenient [13]. Fluoride has also been determined by ion chromatography in water and various other materials [14,15]. This technique is more specific than the spectrophotometric and potentiometric methods. However, the direct determination of fluoride by suppressed conductivity ion chromatography does have problems and the detection is limited to the ppm level [15].

This paper reports a precise, sensitive and simple method for the determination of trace amounts of fluoride in raw materials for pharmaceuticals using gas–liquid chromatography (GLC). This procedure is a modified technique described elsewhere [16–21] for the determination of fluoride in biological fluids. In this chromatographic method, the fluoride ion is converted into an organic derivative by trimethylchlorosilane (TMCS) at acidic pH. The organofluoro derivative is extracted with toluene and determined by GLC with flame ionization detection using isopentane as an internal standard (I.S.). The conversion reactions are as follows [18]:

$$(CH_3)_3 SiCl + H_2O \rightarrow (CH_3)_3 Si OH + HCl$$

$$(CH_3)_3 SiOH + HF \rightarrow (CH_3)_3 Si F + H_2O$$
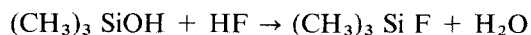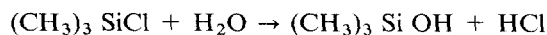
The TMCS is first hydrolysed by water into the corresponding silanol which then reacts with the fluoride ion at acidic pH to form the trimethylfluorosilane (TMFS).

EXPERIMENTAL

Apparatus and materials

A Delsi Model 330 gas chromatograph equipped with a flame ionization detector was used in conjunction with a Servotrace 1-mV recorder. The chromatographic separation was performed using a 2.5 m $\times$ 3 mm (1/8 in.) stainless-steel column packed with 20% DC 200 (methyl silicone fluid) on Chromosorb P-AW, 80–100 mesh (Chrompack). The column was maintained at 100°C and the injection port and detector were operated at 120 and 200°C, respectively. Nitrogen was used as the carrier gas at a flow-rate of 30 ml/min, which developed a head column pressure of about 2.2 bars. The hydrogen and air flow-rates were 40 and 350 ml/min, respectively. The chart speed of the recorder was 5 mm/min. A Maximix Model Vortex (Bioblock) was used for tube agitation. Polyethylene tubes (10 ml) with polyethylene stoppers (Polylabo), resistant to organic solvents, were used for the extraction.

Reagents

TMCS was purchased from Sigma. Isopentane (2-methylbutane) (purissim for gas chromatography), purchased from Fluka and used as the I.S., was diluted 1:2000 (v/v) in TMCS. The I.S. solution was stored at about $-20°C$ in 5-ml vials with PTFE septa and screw caps and was stable for about two weeks. Toluene and concentrated hydrochloric acid were of analytical-reagent grade (Merck) and were stored at about -20 and $+4°C$, respectively.

The stock solution of 100 ppm fluoride was prepared by the dissolution of 221.4 mg of accurately weighed Pharmacopoeia sodium fluoride in distilled water in a 1-l calibrated flask. The standard solution of 10 ppm fluoride was obtained by transferring 10.0 ml of the stock solution to a 100-ml calibrated flask and diluting to volume with water. The fluoride stock and standard solutions were stored in plastic bottles.

Standard calibration

Into six 10-ml polyethylene tubes, 0.05, 0.1, 0.2, 0.5, 1 and 2 ml of 10 ppm fluoride standard solution were added plus a sufficient amount of water to give a final volume of 2 ml corresponding to 0.5, 1, 2, 5, 10 and 20 ppm fluoride. A 100-μl volume of I.S. in TMCS ($-20°C$), 1 ml of toluene ($-20°C$) and 2 ml of concentrated HCl ($+4°C$) were then added. A blank reagent with 2 ml of water and the same reagents was prepared. All reagents were added rapidly and in the specified order. The tubes were stoppered and vortexed for 1 min and then centrifuged for 4 min at 2500 g. A 0.5-ml volume of the organic extract (upper layer) of each standard was immedi-

ately transferred into each of the 3-ml stoppered vials and stored at about $-20°C$ before use. A 5-μl volume of each standard extract was injected into the chromatograph. The standard extracts were stable for about one week if stored at about $-20°C$ in tightly stoppered vials.

Sample preparation

About 100 ± 0.1 mg of the raw material under test was transferred to a 10-ml polyethylene tube and 2 ml of distilled water, 100 μl of I.S. in TMCS ($-20°C$), 1 ml of toluene ($-20°C$) and 2 ml of concentrated HCl ($+4°C$) were added in order. The same procedure was then followed as for the standard calibration. The sample weights vary between 100 and 500 mg depending on the concentration of fluoride in the raw materials.

Calculation

The determination of the fluoride ion in the sample was based on the peak-height ratio of TMFS to the I.S. This is given by a standard curve obtained by dividing the TMFS peak height by the I.S. peak height for each standard. The concentration of the fluoride ion expressed in ppm in the raw material was calculated by the relationship $(C_1 \times 1000)/M$, where C_1 is the concentration of the fluoride ion in the sample, taken from a calibration graph and M is the mass in milligrams of sample taken. The concentration can also be calculated by comparing the TMFS/I.S. peak-height ratio of the sample to that of a single standard containing a known amount of fluoride ion: $C_2 (R_1/R_2) (1000/M)$. In this relationship C_2 is the concentration of the fluoride ion in ppm in a single standard, R_1 and R_2 are the TMFS/I.S. peak-height ratios of the sample and standard, respectively, and M is mass of sample taken in milligrams.

RESULTS

Chromatographic analysis

Typical chromatograms of a sample and a blank reagent are shown in Figs. 1 and 2. The TMFS peak appears first with a retention time about 1.5 min, followed by the I.S. peak (isopentane) with a retention time of about 2 min. The other peaks (trimethylsilanol, TMCS, toluene and all the impurities of these solvents) appeared after TMFS and the I.S.

To obtain the complete elimination of all the parasite peaks, the chromatographic time was about 20 min. No interfering peak was observed with the blank reagent at the retention time of TMFS (Fig. 2).

Analytical variables

Linearity. The standard calibration graphs gave a good linearity for fluoride ions in the range of concentrations tested with a correlation coefficient $r = 1.0002$ and a regression line $y = 0.281x + 0.027$, in which y is the TMFS/I.S. peak-height ratio and x is the concentration of fluoride standards in ppm.

Sensitivity. The detection limit for the assay was 0.01 ppm of fluoride ion in the raw material. As the TMFS peak appears on the chromatogram before the solvent peaks, an adequate sensitivity was obtained by varying the attenuation or the volume of sample injected.

Accuracy. The coefficients of variation (C.V.) determined from replicate analysis ($n = 6$) of two standards (1 and 2 ppm) were 0.6 and 0.7%, respectively. The intra-day C.V. for a sample of raw material (tribasic calcium phosphate) with $n = 6$ was 0.8%.

Recovery. The recovery was studied by a second extraction of about 3.6 ml of the same aqueous phase by 1 ml of toluene plus 100 μl of TMCS without the I.S. No peaks of TMFS or I.S. were observed in the chromatogram after the second extraction of a 2-ppm standard and of a tribasic calcium phosphate sample. The recovery was about 100% for TMFS and the I.S.

Interferences. No peaks of the solvents or their impurities were observed with the reagent blank (Fig. 2) at the retention time of the TMFS peak. It is recommended to wait about 20 min after injection for the complete elimination of all solvent peaks to avoid further interferences.

Interferences were also studied with ions such as Al^{3+}, B^{3+}, Fe^{3+}, Ca^{2+} and Mg^{2+}, for which fluoride has a great affinity. The interference assay was performed as follows: 100 mg of each raw material [aluminium hydroxide, $Al(OH)_3$; sodium borate, $Na_2B_4O_7 \cdot 10H_2O$; boric acid, H_3BO_3; tribasic calcium phosphate, $Ca_3(PO_4)_2$; yellow iron(III) oxide, Fe_2O_3; or magnesium hypophosphite, $Mg(H_2PO_2)_2 \cdot 6H_2O$] were analysed as described earlier. Another 100-mg sample spiked with 20 ppm of fluoride was
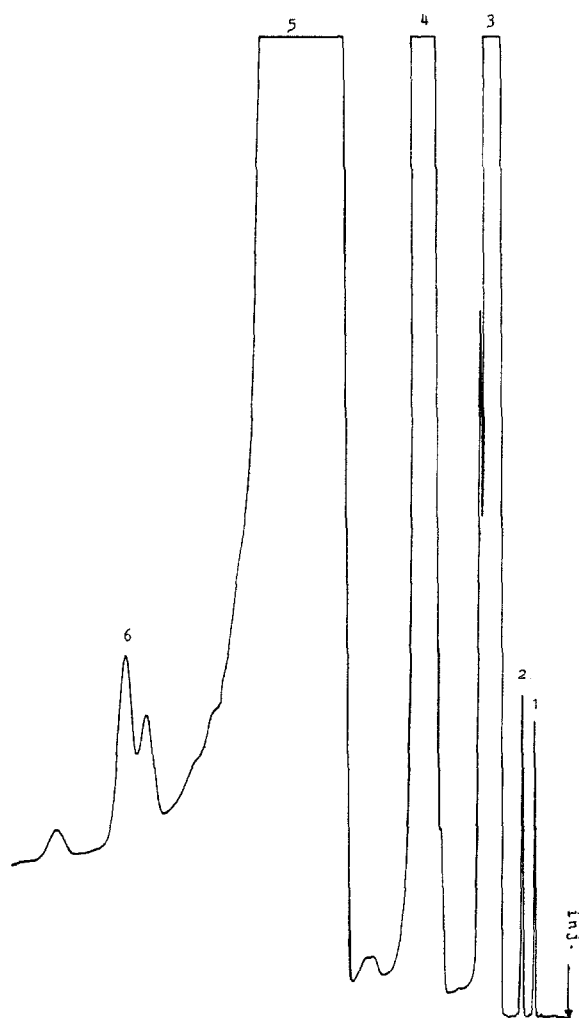
Fig. 1. Chromatogram of a sample tribasic calcium phosphate (pharmaceutical grade). The fluoride level found was 42 ppm. Peaks: 1 = trimethylfluorosilane; 2 = isopentane (I.S.); 3 = trimethylsilanol; 4 = trimethylchlorosilane; 5 = toluene; 6 = solvent impurities.

analysed in parallel. The difference of the TMFS/ I.S. peak-height ratios between the second and the first assay was compared with the peak-height ratio of a 20-ppm standard. The results of this assay are presented in Table I and show no important interference of fluoride with these ions. This means that fluoride has a greater affinity for TMCS than for Al^{3+}, B^{3+}, Fe^{3+}, Ca^{2+} or Mg^{2+} ions.

Raw material studies

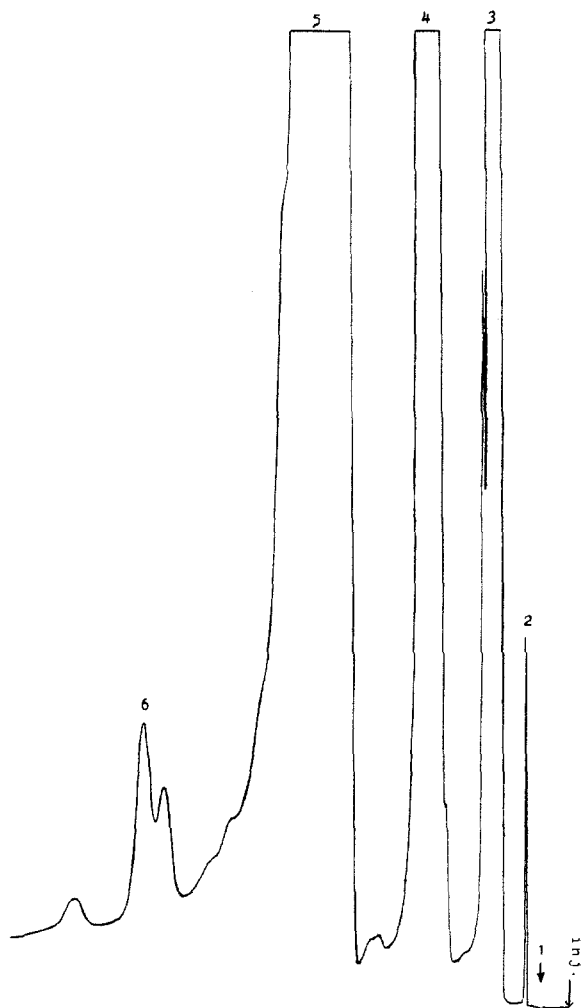Seventeen raw materials used in the pharmaceu-

Fig. 2. Chromatogram of blank reagent. Peaks as in Fig. 1.

tical industry, most of which were calcium salts, were analysed by this method. The fluoride concentrations found in these compounds are presented in Table II. In addition, an inorganic fluoride compound, a sodium monofluorophosphate [FPO $(ONa)_2$] used as raw material in toothpaste, was also determined after dilution of the sample in water (1:10 000). The amount of fluoride found was 13.1%. All previous compounds tested were of pharmaceutical grade, except for one sample of tribasic calcium phosphate which was of technical grade and had a fluoride concentration exceeding the normal values required by the three pharmacopoeia.

TABLE I

INTERFERENCE OF VARIOUS IONS WITH DETERMINATION OF FLUORIDE

Raw material tested	TMFS/I.S. peak-height ratio (mean ± S.D., $n = 6$)			Recovery of 20-ppm F⁻ standard (%)[a]
	First assay (100-mg sample)	Second assay (100-mg sample + 20 ppm F⁻)	20-ppm F⁻ standard	
Aluminium hydroxide	0.74 ± 0.015	5.10 ± 0.12	4.50 ± 0.05	97
Sodium borate	0.34 ± 0.005	4.94 ± 0.14	4.50 ± 0.05	102
Boric acid	0.32 ± 0.005	4.87 ± 0.12	4.50 ± 0.05	101
Calcium phosphate, tribasic	0.94 ± 0.008	5.41 ± 0.10	4.50 ± 0.05	99.5
Iron(III) oxide	0.04 ± 0.002	4.60 ± 0.15	4.50 ± 0.05	101
Magnesium hypophosphite	0.25 ± 0.004	4.66 ± 0.08	4.50 ± 0.05	98

[a] Results are (second assay − first assay)/20-ppm standard.

DISCUSSION

Technical studies

Owing to the high volatility of the TMFS formed and of isopentane used as the I.S. (boiling points 16.4 and 28°C, respectively) [19,21], it is necessary to freeze toluene and the I.S. solution in TMCS at about −20°C before use to avoid the loss of these two compounds during vortex extraction. It is also recommended to store the extracts at about −20°C

TABLE II

CONCENTRATIONS OF FLUORIDE IONS IN RAW MATERIALS USED FOR PHARMACEUTICALS

Raw material tested	Fluoride level (ppm)[a]	Limit values of F ions[b]			
		USP (1990) (%)	European Pharmacopoeia (2nd ed.) (ppm)	British Pharmacopoeia (1988) (ppm)	French Pharmacopoeia (10th ed.) (ppm)
Aluminium hydroxide	32 ± 0.65	ND	ND	ND	ND
Sodium borate	15 ± 0.23	ND	ND	ND	ND
Boric acid	14 ± 0.23	ND	ND	ND	ND
Calcium acetate	18 ± 0.42	0.005	NA	ND	NA
Calcium bromide	25 ± 0.52	NA	NA	NA	NA
Calcium carbonate	38 ± 1.05	0.005	ND	ND	ND
Calcium citrate	6.7 ± 0.21	0.003	NA	NA	NA
Calcium glycerophosphate	10.4 ± 0.35	NA	NA	NA	ND
Calcium phosphate, monobasic	2.6 ± 0.05	NA	NA	NA	NA
Calciumphosphate, dibasic	98 ± 1.10	0.005	100	100	100
Calcium phosphate, tribasic (PG)[c]	42 ± 0.33	0.0075	ND	50	100
Calcium phosphate, tribasic (TG)[c]	162 ± 2.65	0.0075	ND	50	100
Iron(III) oxide	1.7 ± 0.08	ND	NA	NA	ND
Magnesium hypophosphite	11 ± 0.18	NA	NA	NA	NA
Magnesium lactate	5 ± 0.10	NA	NA	NA	NA
Potassium chloride	0.1 ± 0.004	ND	ND	ND	ND
Sodium chloride	0.5 ± 0.01	ND	ND	ND	ND

[a] Results given as mean ± standard deviation; $n = 6$.
[b] ND = Not determined; NA − not available.
[c] PG = pharmaceutical grade; TG = technical grade.

in stoppered vials for the same reasons. The solvents remain as liquid at this temperature and can be used immediately for preparing the sample or for injection into the chromatograph. Yamamoto *et al.* [21] have recommended working in a cold room to prevent evaporation of the solvents. However, this is not always possible in every laboratory and cooling the solvents in a freezer is simpler to achieve. The I.S. can be stored in TMCS for about two weeks instead of being prepared each time as recommended elsewhere [21]. An assay was carried out with all the reagents stored at room temperature or at $+6°C$; the linearity and reproducibility were less satisfactory than at $-20°C$ ($r = 0.922$ and 0.965, C.V.$_s$ = 10.8 and 8.4% for reagents stored at room temperature and $+6°C$, respectively).

Triethylchorosilane (TECS) and tributylchlorosilane (TBCS) were also used for fluoride derivatization. The triethylfluorosilane (TEFS) and tributylfluorosilane (TBFS) formed are less volatile than TMFS and the sample preparation can be performed at room temperature. However, according to Yamamoto *et al.* [21], the reaction time for these derivatization procedures is 90 min instead of 1 min by this technique and many other peaks can appear in the same range as the fluoride derivatization peak. Using the chromatographic conditions described here, TEFS appeared at the same time as the toluene peak (20 min) and TECS at about 40 min; consequently, the chromatographic time using TECS was twice as long as that using TMCS. If TBCS was used under these conditions, TBFS and TBCS were not eluted at $100°C$, but at $200°C$. Using hexane as the extraction solvent and setting the column temperature to $200°C$, many solvent peaks and their impurities appeared before and in the same range as the fluoride derivatization peak using the TECS of TBCS derivatization procedure. These interfering peaks make the fluoride determination difficult and could involve large errors. In contrast, with derivatization by TMCS, no peak was observed before or at the same time as the TMFS and I.S. peaks, as TMFS and I.S. are more volatile than the solvents used in the reaction. Moreover, the slow reaction of TECS or TBCS with fluoride could lead to interferences from Al^{3+}, B^{3+}, Fe^{3+}, Ca^{2+} or Mg^{2+} ions.

As a result of the high reactivity of hydrofluoric acid and fluoride ions on glass to form silicon tetra-

fluoride, the use of polyethylene tubes resistant to organic solvents is necessary for the extraction and storage of the fluoride standards. After about 30 injections, it is recommended that the detector is cleaned and the column heated at $200°C$ overnight to eliminate silyl deposits.

Analytical variables

This chromatographic method has a good accuracy (C.V. about 0.7%), a high sensitivity (0.01 ppm) and good linearity ($r = 1.0002$). It is more rapid, simple and sensitive than the spectrophotometric method used by some European pharmacopoeia. It is also more simple, specific and reproducible than the potentiometric method, described in the USP. In this method, the presence of some metal ions (Al^{3+}, Fe^{3+}), and especially OH^- ions, can interfere with the electrode measurements [22]. In the proposed chromatographic method it was established that Al^{3+}, Fe^{3+}, B^{3+}, Ca^{2+} and Mg^{2+} ions do not interfere because fluoride has a greater affinity towards TMCS than towards these ions (Table I). In addition, the lengthy adjustment period of the electrodes in the low concentration ranges is also a major inconvenience. Some electrodes can cause errors due to excessive drift [13].

Raw material analysis

Table II shows the presence of fluoride ions in all seventeen raw materials tested with a concentration varying between 0.1 and 162 ppm. This variation of fluoride concentrations is due to the presence or absence of this ion in the original minerals and to the manufacture of the raw material. Calcium salts, especially phosphate and carbonate, are the most contaminated by the fluoride ion because most were prepared from ores containing it. The higher fluoride level in tribasic calcium phosphate, technical grade (162 ppm), than in its pharmaceutical grade (42 ppm) has proven the important presence of fluoride in this salt and the necessity for its purification and control in pharmaceutical use. However, the low fluoride level (2.6 ppm) in monobasic calcium phosphate [$Ca (PO_4H_2)_2) \cdot H_2O$] is due to its preparation which consists in heating dibasic calcium phosphate with a strong acid; the fluoride is transformed to hydrofluoric acid which is then eliminated by evaporation.

Some other organic calcium salts such as calcium

citrate (6.7 ppm), calcium glycerophosphate (10.4 ppm) and calcium acetate (18 ppm) contain less fluoride than inorganic calcium salts such as phosphates or carbonates because these synthetic compounds are generally obtained by the condensation of inorganic calcium salts with their organic acid; the fluoride present in the inorganic raw materials could be partially eliminated either by purification of the synthetic organic compound formed or by the evaporation of the hydrofluoric acid if the synthesis reaction is sufficiently acid. The lower fluoride concentrations in magnesium lactate (5 ppm) than in magnesium hypophosphite (11 ppm) could be explained by these reasons.

The traces of fluoride in potassium chloride (0.1 ppm) are explained by the purity of this ore. Sodium chloride contains more fluoride (0.5 ppm) because it is generally obtained from sea water containing about 1 ppm fluoride [2]. The presence of large amounts of fluoride (32 ppm) in aluminium hydroxide is explained by the use of calcium fluoride as a flux in aluminium metallurgy. No limit value for the concentration of fluoride in aluminium hydroxide is given in any pharmacopoeia. In the USP, many descriptions of calcium salts (Table II) require a limit of fluoride ions, contrary to the European pharmacopoeia in which an assay is required for only two compounds of calcium phosphate. In this work, 38 ppm of fluoride were found in calcium carbonate.

The proposed chromatographic method determines total inorganic fluorides, including water-soluble and insoluble forms, as do the two techniques cited previously. Most fluorides in calcium salts are in the form of calcium fluoride. This may be less soluble in the gastrointestinal tract than sodium fluoride and consequently less toxic. According to Whitford [23], the gastrointestinal absorption of calcium fluoride in food is about 63%, compared with 90% for sodium fluoride. Ericsson [24] reported that certain studies show the formation of various complex ions such as $(CaF)^+$ or $(MgF)^+$ which are more soluble and are susceptible to metabolism in humans. Calcium fluoride can be as toxic as sodium fluoride if the amount ingested is large [2]. This explains the endemic fluorosis observed in the Darmous population (Morocco) who absorbed calcium fluoride present in food, air and water.

The determination of the fluoride ion in inorganic raw materials for pharmaceuticals is necessary because of the easy contamination of these compounds during manufacture and the potential toxicity of the fluoride ion. The simple, rapid and accurate gas chromatographic method proposed should be developed in the pharmaceutical industry for the routine control of trace amounts of fluoride.

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