Determination of Organically Bound Fluorine Using Sodium Biphenyl Reagent and a Fluoride-Specific Ion Electrode

B. C. JONES, J. E. HEVERAN, and B. Z. SENKOWSKI

Abstract ☐ A simple, accurate, and specific method for direct determination of carbon-bonded fluorine is described for various aromatic compounds of pharmaceutical interest, containing as little as 4% fluorine. Sodium biphenyl reagent was utilized for quantitative liberation of bound fluorine; a fluoride-specific ion electrode was used, in conjunction with a high ionic strength buffer solution, for direct measurement of the liberated fluoride. Incorporation of a subsequent "oxidation" step, using hydrogen peroxide, resulted in quantitative determination of fluorine for certain organo-fluoro compounds which were difficult to ionize completely using a modified oxygen flask combustion technique. It was possible to determine fluorine routinely for up to 20 samples in an 8-hr. period. Precision and accuracy studies are included.

Keyphrases ☐ Fluorine, organically bound—determination ☐ Oxidation with hydrogen peroxide—fluorine analysis ☐ Sodium biphenyl reagent—bound fluorine liberation ☐ Fluoride-specific ion electrode—organically bound fluorine determination

Many suitable methods are available for determining organically bound fluorine. These methods involve ionization of the bound fluorine using: sodium in liquid ammonia (1, 2); sodium peroxide fusion (3, 4); sodium or potassium fusion (5–9); combustion (10–30); "reductive cleavage" with a stabilized, highly reactive, ether–sodium–aromatic hydrocarbon complex (31–34); and potassium hydroxide in dimethyl sulfoxide (35). The ionization is followed by colorimetric, gravimetric, titrimetric, potentiometric, or fluoride-specific ion electrode measurement of the liberated fluoride (36–40).

Difficulties generally encountered when the widely used oxygen-flask combustion method is utilized for liberation of the bound fluorine include: incomplete combustion, loss of liberated fluoride, and loss of accuracy for compounds having low fluorine content. Consequently, the oxygen-flask combustion method may not be generally suitable for routine determination of bound fluorine in a significant number of pharmaceutical substances with the precision and accuracy expected for compendia methods.

Of the various alternate ionization procedures, reductive cleavage with a stabilized, highly reactive, ether–sodium–aromatic hydrocarbon complex appeared most suitable for rapid, convenient, and accurate liberation of the bound fluorine. These complexes have been utilized for rapid decomposition of organic halogens, including those containing fluorine (41–46). Reportedly, the most effective complex in terms of stability, reactivity at room temperature, and active sodium concentration is the diphenyl–sodium–dimethoxy–ethane complex (sodium biphenyl reagent).

This report describes the conditions generally applicable for the quantitative ionization of bound fluorine in pharmaceutical substances with sodium biphenyl reagent and for the subsequent rapid determination of the liberated fluoride with a fluoride-specific ion electrode.

EXPERIMENTAL

Reagents—The following were used: sodium biphenyl reagent (premixed solution in 15-ml. vials)[1]; ethylene glycol dimethyl ether, b.p. 83–85°[2]; spectroquality dimethyl sulfoxide[2]; certified tetrahydrofuran[3]; reagent grade hydrogen peroxide (30%)[3]; certified ACS grade sodium chloride[3]; certified ACS grade 2-propanol[3]; ACS reagent grade glacial acetic acid[4]; analytical reagent grade sodium hydroxide[5] and sodium citrate[6]; analytical reagent grade sodium fluoride[6]; and 1 M sodium hydroxide USP.

2-Propanol Solution (59% v/v)—Dilute 590 ml. of 2-propanol to 1 l. with demineralized water. Mix and allow the solution to cool to room temperature. Adjust final volume to 1 l. with demineralized water and mix well.

Alcoholic Acetate Buffer Solution—In a 2-l. volumetric flask, dissolve 110 g. of sodium chloride and 1 g. of sodium citrate in 600–800 ml. of demineralized water. Add and dissolve 150 g. of sodium hydroxide. Cool to room temperature and, with stirring, cautiously add 450 ml. of glacial acetic acid. Cool to room temperature, add 600 ml. of 2-propanol, and dilute to volume with demineralized water. The pH of this solution should be 5–5.5 with an ionic strength of 4 M.

Standard Fluoride Stock Solution (1.0 mg. Fluoride/ml.)—Weigh 2.211 g. of sodium fluoride, previously dried at least 24 hr. in an oven at 100–110°, into a 1-l. volumetric flask and dissolve in 200 ml. of demineralized water. Add 1.0 ml. of 0.1 N NaOH and dilute to volume with demineralized water.

Working Standard Fluoride Solution—Dilute the stock fluoride solution with alcoholic acetate buffer solution to prepare solutions equivalent to 0.02, 0.05, 0.10, 0.15, and 0.20 mg. of fluoride/100 ml. Add 10 ml. of reagent blank, as prepared for the respective assay procedure, to each working standard solution before final dilution. Prepare fresh solutions for each analysis.

Apparatus—An Orion fluoride-specific ion electrode, model 94-09, in conjunction with a modified Sargent (glass-sleeve) calomel reference electrode, model S-30084-15, was used with an Orion model 801 research digital pH meter for potential measurements. The reference electrode was modified with a mixture of 70 ml. of saturated KCl solution (freshly prepared or precooled to at least 9°) and 30 ml. of 2-propanol. The electrode was filled with the clear supernatant solution and conditioned in the 2-propanol–saturated KCl solution (30:70) for a minimum of 2 hr. before use.

Assay Procedure—*Sample Preparation*—Type A compounds (soluble without heating, requiring little or no shaking for 0.1–0.2% solubility, w/v)—Accurately weigh a sample equivalent to 16 mg. of fluorine, dissolve in 70–80 ml. of tetrahydrofuran or ethylene glycol dimethyl ether, and dilute to 100 ml. Pipet exactly 15 ml. of the solution into a 200-ml. volumetric flask. Add the contents of a 15-ml. vial of sodium biphenyl reagent and mix. After 5–10 min., destroy the excess reagent with 5–10 ml. of 2-propanol and dilute to volume with 2-propanol. Dilute 10.0 ml. of this solution to 100 ml. with alcoholic acetate buffer solution.

Prepare a reagent blank by diluting 15.0 ml. of sample solvent and 15 ml. of sodium biphenyl reagent to 200 ml. with 2-propanol (as

the liberated fluoride with a fluoride-specific ion electrode.

per sample preparation). Use this solution in preparing working standard fluoride solutions.

Type B compounds (soluble with heating and/or prolonged shaking for 0.1–0.2% solubility, w/v)—Accurately weigh a sample equivalent to 20 mg. of fluorine; dissolve in 70–80 ml. of tetrahydrofuran or ethylene glycol dimethyl ether and dilute to 100 ml. (Compounds that were not readily soluble in tetrahydrofuran or ethylene glycol dimethyl ether were predissolved in 5.0 ml. of dimethyl sulfoxide with heating on a steam bath and diluted to 100 ml. with tetrahydrofuran or ethylene glycol dimethyl ether.) Pipet a 15-ml. aliquot of sample solution into a 500-ml. round-bottom flask, add the contents of a 15-ml. vial of sodium biphenyl reagent, and mix. After letting the flask stand at room temperature for 5–10 min., add 50.0 ml. of 2-propanol, 10.0 ml. of 30% H_2O_2 (reagent grade), and 4.0 ml. of 1 M NaOH, respectively. Connect the flask to a clean, dry, water-cooled condenser and reflux, using a preheated hot plate set for medium heat (about 250°), until the H_2O_2 is completely destroyed. (This is indicated by irregular boiling with bumping and cessation of rapid boiling and usually requires 50–60 min.) Cool the sample to room temperature, quantitatively transfer to a 250-ml. volumetric flask, and dilute to volume with 59% (v/v) 2-propanol. Dilute 10.0 ml. of sample solution to 100 ml. with alcoholic acetate buffer solution.

Prepare a reagent blank by pipeting 15 ml. of sample solvent into a 500-ml. round-bottom flask and treating in the same manner as the sample. For samples predissolved with dimethyl sulfoxide, prepare the reagent blank by diluting 5 ml. of dimethyl sulfoxide to 100 ml. with the sample solvent. Transfer 15.0 ml. of this solution to a 500-ml. round-bottom flask and treat in the same manner as the sample. After diluting to 250 ml. with 59% (v/v) 2-propanol, use this solution in preparing the working standard fluoride solutions.

Potential Measurements—Transfer the solution to be measured to a 150-ml. beaker containing a Teflon-coated stirring bar. (Although plastic labware is not required, it is recommended, particularly for sample containment during potential measurements.) Immerse the specific fluoride and modified reference electrodes, and measure the millivolt potential with constant stirring. Use an electric stirrer with an insulated top or with an asbestos pad to reduce heat transfer to the stirred solution. Take a final reading when the potential has stabilized within ±0.1 mv. or allow 1–2 min. before each measurement. Measure the working standard solutions beginning with the lowest fluoride concentration, i.e., 0.02 mg./100 ml.

Plot fluoride concentration (mg./100 ml. *versus* millivolts for each working standard solution on semilogarithmic paper (1 cycle). The fluoride concentration in the measured sample solution is determined directly from this graph.

Alternately, the potentials observed for the standard solutions can be used to obtain a least-squares line represented by the equation:

$$y = mx + b \qquad \text{(Eq. 1)}$$

where y = potential in millivolts, x = log of fluoride concentration in mg./100 ml., m = slope, and b = intercept.

The potential obtained for the sample solution is substituted in Eq. 1, and the equation is solved for x. The antilog of x equals the fluoride concentration of the measured sample solution.

Single-Point Standard Comparison—If the slope of the concentration curve remains constant within ±2 mv., the standard curve or the corresponding slope (m) and intercept (b) values may be used as a fixed calibration for single-point reference standardization as follows. Prepare a standard fluoride solution as previously described, equivalent to 0.10 mg. fluoride in 100 ml. With an Orion digital pH meter or equivalent, set the dial (functional switch) in the relative millivolt position and measure the potential of this solution as previously described. Adjust the meter, using the calibration control to read the exact potential (y), calculated from Eq. 1 or read directly from the fluoride concentration curve.

To obtain the fluoride concentration in the measured sample solution, take the antilog of x calculated for the corresponding y using Eq. 1 or read fluoride concentration directly from the standard curve.

RESULTS AND DISCUSSION

Sample Dissolution—The choice of sample solvents was limited to those that were water miscible, did not cause instant decomposi-
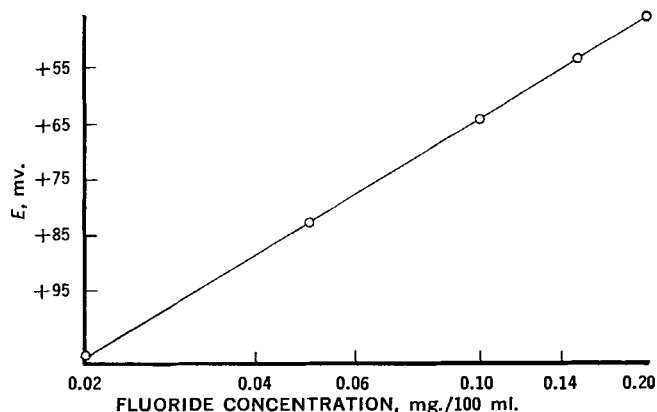

Figure 1—*Standard fluoride calibration curve.*

tion of the sodium biphenyl reagent, and had at least a 0.1% sample solubility by weight. Of the various solvents examined, tetrahydrofuran, ethylene glycol dimethyl ether, and dimethyl sulfoxide were found to be suitable. Most of the compounds tested were readily soluble in dimethyl sulfoxide. However, the sodium biphenyl reagent was slowly decomposed by dimethyl sulfoxide, and fluorine recoveries were not quantitative. Therefore, compounds that required heating to attain at least 0.1% solubility by weight in tetrahydrofuran or ethylene glycol dimethyl ether were first dissolved in a small amount of dimethyl sulfoxide and then diluted with tetrahydrofuran or ethylene glycol dimethyl ether.

Sodium Biphenyl Reaction—Although the mechanism of the reaction is not well documented, it is believed that decomposition of organo-fluoro compounds with sodium biphenyl reagent involves reductive cleavage of the bound fluorine (31, 37). The reaction is apparently instantaneous at room temperature, since no significant differences in fluorine recoveries were observed for reaction periods of 1–30 min. Although the reaction between sodium biphenyl reagent and the various organo-fluoro compounds was apparently instantaneous, the reaction time for this procedure was standardized at 5–10 min.

For the 12 compounds examined, the need for subsequent oxidation for quantitative fluorine recoveries was apparently not structurally dependent. However, the relative solubility of the compounds in the sample solvents appeared to be a determining factor in the need for subsequent oxidation. All compounds that did not require subsequent oxidation for quantitative fluorine recoveries were readily soluble in the sample solvents (soluble without heating and requiring little or no shaking for 0.1–0.2% solubility, w/v). Correspondingly, six of the nine compounds that required subsequent oxidation were difficult to dissolve in the sample solvents.

"Oxidation" of Reaction Mixture with Hydrogen Peroxide—For most of the compounds tested, it was necessary to "oxidize" the reaction mixture with hydrogen peroxide to obtain quantitative fluorine recoveries. The oxidation step involved the addition of an excess of hydrogen peroxide to the reaction mixture after destroying the excess sodium biphenyl with 2-propanol. A small amount of sodium hydroxide was added to the mixture to ensure basic pH conditions, and the entire reaction mixture was refluxed until the hydrogen peroxide was completely destroyed. This usually required about 1 hr. of total refluxing time. Since hydrogen peroxide was a sufficient oxidant for all compounds investigated, no other oxidants were tested.

When subsequent oxidation was required for quantitative fluorine recovery after reaction of the compound with sodium biphenyl reagent, it was suspected that an insoluble fluoro-reaction product or an insoluble sodium compound was formed (42, 44). It is also possible that biphenyl fluoride was formed; however, this seems improbable since acidic reaction conditions would be necessary for this reaction to take place (47).

Dilution of Reaction Mixture—The oxidized reaction mixture was diluted with 59% (v/v) 2-propanol. When ethylene glycol dimethyl ether was used as the sample solvent, the 2-propanol-water ratio of the solution, used to dilute the oxidized reaction mixture, was critical. A variation of less than ±1% in the indicated ratio resulted in either precipitation or biphase formation in the resultant sample solution. However, this was not the case when tetrahydrofuran or

Table I—Determination of Organically Bound Fluorine in Various Organo-Fluoro Compounds

Compound	Empirical Formula	Fluorine Found, %	Theoretical Fluorine, %	Ratio Recovered, %	Absolute Error, %	Standard Deviation	Precision[a]
5-Fluorouracil	$C_4H_3FN_2O_2$	14.52	14.6	99.5	0.08	0.62	1.43
Flurazepam hydrochloride	$C_{21}H_{23}ClFN_3O \cdot$ 2 HCl	4.14	4.12	100.5	0.02	0.45	1.25
Chloro-fluoro	$C_{21}H_{23}ClFN_3O$	4.93	4.90	100.6	0.03	0.42	1.02
Nitro	$C_{16}H_{12}FN_3O_3$	6.07	6.07	100.0	0.00	0.16	0.39
Chloro-fluoro	$C_{17}H_{14}ClFN_2O_2$	5.72	5.71	100.2	0.01	0.29	0.80
Chloro-fluoro HCl salt	$C_{17}H_{14}ClFN_2O_2 \cdot$ HCl	5.16	5.15	100.2	0.01	0.61	1.49
Chloro-fluoro HCl salt	$C_{10}H_{11}ClF_3N \cdot HCl$	20.82	20.79	100.2	0.03	0.33	0.92
Chloro-fluoro H₂SO₄ salt	$[C_{10}H_{11}ClF_3N]_2 \cdot$ H_2SO_4	19.71	19.88	99.2	0.17	0.32	0.82
5-Fluorouridine	$C_9H_{11}FN_2O_6$	7.17	7.25	99.0	0.08	0.25	0.65
Dihydroxy-fluoro	$C_9H_{11}FN_2O_5$	7.75	7.72	100.4	0.03	0.34	0.86
Amino	$C_4H_4FN_3O$	14.69	14.72	99.8	0.03	0.33	0.76
Bromo-fluoro	$C_{10}H_{14}BrFN_2O_6$	5.32	5.32	100.0	0.00	0.42	1.08
Average					0.04	±0.38	±0.96

[a] Based on four to eight determinations per compound at the 95% confidence level.

dimethyl sulfoxide was the sample solvent. Samples that did not require subsequent oxidation after the sodium biphenyl reaction were diluted directly with 2-propanol.

Choice of Buffer Solution—To determine the liberated fluoride directly, without separation from the reaction mixture, a high ionic strength buffer system which was compatible with the reaction mixture was required. It had previously been determined that the optimum pH range for fluoride determination in the $10^{-4}-10^{-5}$ M range was 4.5–8.0 (48). Therefore, various alcoholic buffer systems covering the desired pH range were used to dilute the oxidized reaction mixture from 15 to 100 ml. Methanolic and ethanolic buffer systems were not suitable, since precipitation occurred even when 40% alcoholic buffer systems were used to dilute the sample solution. However, it was found that the alcoholic acetate buffer solution containing sodium chloride (pH 5–5.5) was compatible with the reaction mixture. A uniform solution resulted when the sample solution was diluted as much as 1 to 4 with this buffer solution, and the pH of this solution was equal to the initial buffer solution.

Electrode Response and Potential Measurements—Electrode response to fluoride ion was linear throughout the working range of $10^{-4}-10^{-5}$ M or 0.02–0.20 mg. F⁻/100 ml. (Fig. 1). The slope of the response curve was 58.1 mv. Electrode response was rapid and, in most cases, equilibrium was reached in less than 30 sec. in stirred solutions. Since potential measurements were made in an alcoholic solution, the type and condition of the reference electrode was a critical factor. Excessive electrode drift, exceeding 0.5–1 mv./min., was encountered when measurements were made using a conventional calomel reference electrode. Therefore, it was necessary to use a modified calomel reference electrode. Potential measurements were reproducible within ±0.1 mv. and were relatively drift-free with the modified reference electrode.

Sample Analysis—The average standard deviation, based on percent sample fluorine, was ±0.38% for 12 organo-fluoro compounds containing from 4 to 21% organically bound fluorine. The average absolute error, assuming 100% purity, was less than 0.17% for all compounds tested (Table I).

CONCLUSION

The combination of the sodium biphenyl reagent to liberate bound fluorine and a fluoride-specific ion electrode for direct determination of the ionized fluoride resulted in a convenient and accurate method for determining bound fluorine in various organo-fluoro compounds of pharmaceutical interest.

By using the developed method, it was possible to determine accurately fluorine for 8–10 different samples in an 8-hr. period. For compounds that did not require subsequent hydrogen peroxide oxidation, a single analyst could analyze 16–20 samples in an 8-hr. period.

Although only a limited number of organo-fluoro compounds containing carbon–fluorine bonds were tested, it is believed that the method can be used to determine organically bound fluorine

routinely in other types of organo-fluoro compounds. The method should prove especially advantageous when erratic results, due to incomplete sample combustion, are obtained when using one of the more conventional means of fluorine ionization.

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Drug-Absorption Analysis from Pharmacological Data II: Transcorneal Biophasic Availability of Tropicamide

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Abstract ☐ Following an ophthalmic dose, the mydriatic drug, tropicamide, may traverse the cornea to reach its vicinal sites of action (biophase) directly or indirectly gain access subsequent to its systemic absorption. The latter route of entry into the biophase may be attributed to fluid volume loss and scleral absorption. A means by which the relative quantities of the drug absorbed transcorneally directly into the biophase and indirectly following systemic absorption at any time can be discerned from concomitant measurements of pupillary diameters in drug-treated and untreated eyes is described. However, except at very high dosage, the observed magnitude of effects in the control eye were insufficient for this method to be practical for tropicamide. The determination of the total relative quantities of drug ultimately dissipated from the absorption site by routes other than transcorneal absorption into the biophase was, however, approximated from measurements performed on the treated eye alone. Semilogarithmic plots of the time course of transcorneal drug passage were linear, indicating the biophasic availability of tropicamide to occur through the operation of apparent first-order processes. A comparison of pharmacological and biokinetic parameters characterizing the mydriatic behavior of tropicamide administered in vehicles having a pH of 5.0 and 7.4 is presented.

Keyphrases ☐ Tropicamide mydriasis—transcorneal biophase parameters, vehicle pH influence, rabbits ☐ Pharmacokinetic parameters, biophase—transcorneal tropicamide mydriasis, vehicle pH influence ☐ Mydriatic response behavior, tropicamide—vehicle pH influence, rabbits

The influence of formulation factors on such pharmacological response characteristics as onset and duration of response, peak response intensity, time of peak response, and rate(s) of dissipation of effect is an important consideration in the development and evaluation of pharmaceutical products. A drug induces its biological effects when it enters its biophase compartment where it interacts with its receptor sites. The rates at which a drug enters and is subsequently dissipated from its biophase determine the time course of the induced response intensity and, therefore, the characteristics of its pharmacologic behavior. At the two extremes, the availability of a drug to its biological sites of action is either limited by its release from its dosage form or by such biological factors as the permeability of tissue barriers, metabolism, distribution of the drug into tissue depots, and excretion. When the drug is administered by other than parenteral routes, its biophasic availability, *i.e.*, the total quantity of drug that has penetrated to the biophase at any time, may also be severely affected by peripheral losses from the site(s) of absorption. The relative quantities of drug that are absorbed to contribute subsequently to the pharmacologic effect and the quantities that are peripherally eliminated from the site of administration are dependent upon the relative rates of the competing, simultaneously operative processes.

As is commonly the case with ophthalmic preparations, drugs are generally most rapidly available for absorption when administered in the form of their aqueous solutions. The composition of such solutions can influence the rates at which the drug becomes available to its biophase because it can determine the form of the drug and, therefore, its tissue permeability. Alternatively, the vehicle may exert an influence directly on the permeability properties of the tissue barriers which the