[Chem. Pharm. Bull. 21(8)1632—1640(1973)]

UDC 547.551.2.08 : 615.31.033

Applications of Indirect Atomic Absorption Analysis of Drugs by Forming Complexes.[1] II.[2] Determination of Flufenamic Acid in Perfusion Experiment using the Rat and Pharmaceutical Preparations

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(Received October 16, 1972)

In the presence of Cu and 2-(2-hydroxyethyl)pyridine, flufenamic acid formed a chelate compound, which could be extracted with propyl acetate. The amount of flufenamic acid was obtained indirectly by measuring Cu in this propyl acetate solution by atomic absorption. When this method was applied to the perfusion of the rat small intestine and to the analysis of preparations, good results were obtained without any interference. It was also found that flufenamic acid, Cu, and 2-(2-hydroxyethyl)pyridine combined in the ratio of 1 to 1 to 1 to form a chelate.

Atomic absorption analysis is originally a very convenient method for determination of metals. Some drugs, such as cyanocobalamin, copper chlorophyllin sodium, and calcium pantothenate, contain metals, which can be determined by the atomic absorption method. There are many drugs with groups able to form chelate compounds with metals, though they do not contain metals in their molecules. One of the authors, who noted this fact, analyzed butylscopolamine bromide and butylscopolamine tannate, parasympathetic nerve blocking agents, in blood, urine, and feces in the following way.[2] Butylscopolamine bromide and butylscopolamine tannate formed complex compounds with cobalt in an acidic medium in the presence of tartaric acid. The complex compounds thus formed were soluble in chloroform and cobalt in the organic solvent was determined by the atomic absorption method. The values obtained by this method agreed well with those obtained by using a labeled compound.[4] After the authors reported this method, Kidani, *et al.*[5] reported the atomic absorption analysis of chinoform, which had a group able to form an oxine-type chelate compound, after extracting its zinc chelate with methyl isobutyl ketone (MIBK). Except for report by Minamikawa, *et al.*[2,4] and by Kidani, *et al.*,[5] there are no other reports on the analysis of drugs, which have no metals but have chelate forming groups in their molecules, by the atomic absorption method after chelate formation, as far as the authors are aware.

As a serial study of this kind, we noted the new fact that flufenamic acid, a non-steroid antiphlogistic, formed a chelate compound with copper quantitatively in the presence of 2-(2-hydroxyethyl)pyridine and established an indirect method for determination of flufenamic acid in biological samples and preparations by extracting the chelate compound with propyl acetate, and determining copper in the organic solvent by atomic absorption analysis.

An ultraviolet absorption method[6] and a fluorometric analysis[7] have been used for the determination of flufenamic acid. The former method, however, was interfered by Phenol

1) This paper is Part VII of a series entitled "Studies on the Quality of Pharmaceutical Preparations"; Part VI: T. Minamikawa, T. Yoshida, K. Sakai, and Y. Nakabasami, *Eisei Kagaku*, **19**, 209 (1973).
2) T. Minamikawa, K. Matsumura, A. Kamei, and M. Yamakawa, *Bunseki Kagaku*, **20**, 1011 (1971).
3) Location: *Inokuchi, Katsuyama, Fukui-ken, 911 Japan.*
4) T. Minamikawa, K. Matsumura, and A. Kamei, *Clin. Rep.*, **5**, 617 (1971).
5) Y. Kidani, K. Inagaki, Y. Nakane, and H. Koika, "Abstr. Papers 91st Annu. Meet. Pharm. Soc. Japan," 1971, p. 517.
6) H. Maruyama, E. Fujihira, and M. Nakazawa, *J. Med. Soc. Toho Japan*, **16**, 558 (1969).
7) *a*) Y. Hattori, T. Arai, T. Mori, and E. Fujihira, *Chem. Pharm. Bull.* (Tokyo), **18**, 1063 (1970); *b*) G. Devaux, P. Mesnard, and A.M. Brisson, *Ann. Pharm. Fr.*, **27**, 239 (1969).

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Red which was added to samples of perfusion experiments for water adjustment. The latter was also affected by water[7a] and had poor quantification,[7b] though its sensitivity was high. A method using thin-layer chromátography[8] was too slow for analysis of many samples. A gas chromatographic method[9] was lower in sensitivity than the ultraviolet absorption method and besides it was necessary to convert samples to their derivatives. In contrast, the atomic absorption method developed by the present authors had higher sensitivity than the thin-layer chromatographic method, could be applied to biological samples and preparations without any separation procedures, and was not affected by water.

Experimental

Apparatus and Reagents——Atomic absorption spectrophotometer: Hitachi-Perkin Elmer Model 303, Hitachi hollow cathode lamp (for a single element) as the source of light, chart readout as an accessory, and Hitachi recorder Model QPD_{73} were used. Spectrophotometer: Hitachi autorecording spectrophotometer Model EPS-3T and Hitachi Model 139 spectrophotometer. Gas chromatograph: Hitachi Model K-23, 1.5% SE-30 (Chromosorb G, 60—80 mesh, acid washed, treated with dimethylchlorosilane), 1 m×3 mm glass tube, injection temp. 235°, N_2 flow rate 40 ml/min, H_2 1.5 kg/cm^2, F.I.D. were used. Although the above conditions were the same, oven temp. was 160° for analysis of flufenamic acid in complex, and 95° for analysis of 2-(2-hydroxyethyl)pyridine. Ultracentrifuge: Kubota Model K-80. Shaker: Iwaki Model KM all-purpose shaker VD. pH Meter: Toa Dempa Model HM-5A. Pump with constant flow: Toko Kagaku Seiki Model CV-2, Sanyo Rikagaku Kiki Model T-63.

Flufenamic Acid (for Standard): mp 134.3°, recrystallized from EtOH. A value obtained by neutralization determination was 100.4%.

Propyl Acetate: 1 liter of propyl acetate (Kanto Kagaku) was washed with 500 ml of $NaHCO_3$ solution (1→100) and then with water till it became neutral, desiccated with active $CaSO_4$, and filtered.

Copper-pyridine-ethanol Reagent: 9.0 ml of 0.1% $CuSO_4$ solution was added to 1.5 ml of 2-(2-hydroxyethyl)pyridine (slightly yellow liquid d_4^{20}: 1.098, n_D^{20}: 1.536. Purity (gas chromatograph) min. 99%.[10] Tokyo Kasei). this reagent was prepared before use. Other chemicals used were of analytical grade.

Selection of Pyridine Derivatives——Compounds, which have $-NH_2$ and $-COOH$ as chelate forming groups, like flufenamic acid, have smaller stability constants compared to those with oxine-type chelate

Table I. Colour Reaction with Pyridine Derivatives and Flufenamic Acid

No.	Compounds	Colour of chloroform layer	
		Sample[a]	Blank[b]
1	Pyrazole	pale indigo	white turbidity
2	Quinolinic acid	white turbidity	white turbidity
3	Pyridine-3-4-dicarboxylic acid	white turbidity	white turbidity
4	Isonicotinic acid	pale indigo	white turbidity
5	Picolinic acid	white turbidity	white turbidity
6	Pyrazine	indigo	white turbidity
7	Pyridine-3-sulfonic acid sodium salt	pale indigo	white turbidity
8	2-Hydroxymethylpyridine	green	white turbidity
9	2-(2-Hydroxyethyl)pyridine	dark green	white turbidity
10	Lutidinic acid	white turbidity	white turbidity
11	Pyridine-2-5-dicarboxylic acid	white turbidity	white turbidity
12	Nicotinic acid	white turbidity	white turbibity
13	2-Pyridinealdehyde	colourless	white turbidity
14	Pyridine	yellowish green	white turbidity

a) flufenamic acid-0.1N NaOH (saturated) 2 ml+0.1% $CuSO_4$ solution 2 ml+each pyridine derivative (saturated water solution, if solide, original liquid, if liquid) 2 ml+chloroform 5 ml

b) 0.1N NaOH 2 ml+0.1% $CuSO_4$ solution 2 ml+each pyridine derivative (saturated water solution, if solide, original liquid, if liquid) 2 ml+chloroform 5 ml

8) H.D. Dell and R. Kam, *Arch. Pharm.*, 303, 785 (1970).
9) K. Yamashita, K. Goromaru, S. Goto, H. Ichihara, and S. Iguchi, "Abstr. Papers 89st Annu. Meet. Pharm. Soc. Japan," 1969, p. 102.
10) From specification of Tokyo Kasei; Pyridine ethanol, their name for 2-(2-hydroxyethyl)pyridine.

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forming groups, and their chelate compounds are difficult to extract with organic solvents[11] as they have polar groups in their molecules. Anthranilic acid,[12] a fundamental skeleton of flufenamic acid, forms a chelate compound hardly soluble in water with Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, *etc.*, and is used as a reagent for gravimetric analysis of metals as their solubility product is very small. An attempt has been made to analyze a ferric ion by utilizing the fact that this ion forms a chelate compound with anthranilic acid, which could be extracted with an organic solvent.[11] Details of this method were not reported, but, the range of the standard curve might be limited and reproducibility might be poor. It was noted that chelate compounds formed by adding copper and some pyridine derivatives to flufenamic acid could be extracted with organic solvents, and examinations were made on chelate formation with various pyridine derivatives under the conditions shown in Table I. Following experiment was then carried out pyridine derivatives which showed different color from a blank. To a mixture of 3 ml of 0.1% $CuSO_4$ solution and 4 ml of 100 μg/ml solution of flufenamic acid (0.001N NaOH solution), 3 ml each of various pyridine derivatives (Nos. 1, 2, 6, 7, 8, 9, and 14 in Table I) was added and the mixture was extracted with propyl acetate so that the concentration of flufenamic acid became 20 μg/ml, and atomic absorption of the solution thus obtained was determined. Among the pyridine derivatives used, pyridine was not desirable as its blank value was high when the concentration of flufenamic acid became low. 2-(2-Hydroxyethyl)pyridine, showed absorption 5 times as high as the other pyridine derivatives and it was used in the following experiments.

Selection of Solvents for Extraction——The most desirable solvent for extraction of a chelate compound formed with flufenamic acid and copper-pyridine-ethanol reagent was examined. The chelate compound was not extracted with BuOH, heptane, iso-BuOH, diethyl phthalate, ether, toluene, xylene, and benzene. Methyl isobutyl keton, dichloroethane, diethylamine, AcOBu, amyl acetate, and CCl_4 were not desirable as the reagent blank was also extracted by them. With propyl acetate, 3% isoamyl alcohol-heptane solution and AcOEt, however, only the chelate compound was extracted, and propyl acetate was chosen because of its high absorption.

Examination on the Amount of Reagents to be used——(1) Amount of Copper Sulfate Reagent: To a mixture of 1.5 ml of 2-(2-hydroxyethyl)pyridine and 4 ml of 100 μg/ml solution of flufenamic acid (0.001N NaOH solution), 0 to 15.0 ml of 0.1% $CuSO_4$ solution was added, the mixture was shaken and extracted with propyl acetate so that the final concentration of flufenamic acid became 20 μg/ml and absorbance of the extract solution was measured. As absorbance was almost constant with 8 to 15 ml of 0.1% $CuSO_4$ solution, 9 ml of the reagent was used in the following experiments.

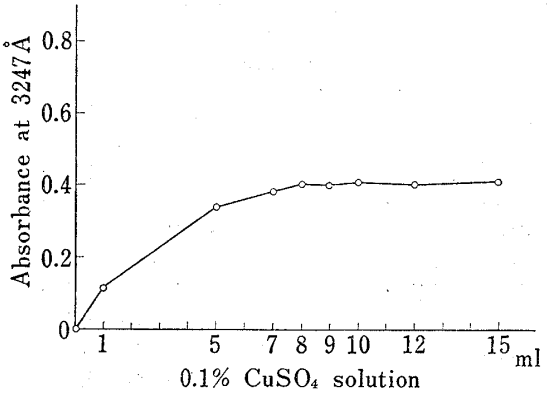

Fig. 1. Effect of Concentration of 0.1% $CuSO_4$ Solution

flufenamic acid: 400 μg
2-(2-hydroxyethyl)pyridne: 1.5 ml
recorder readout scale: X1
meter response: X1

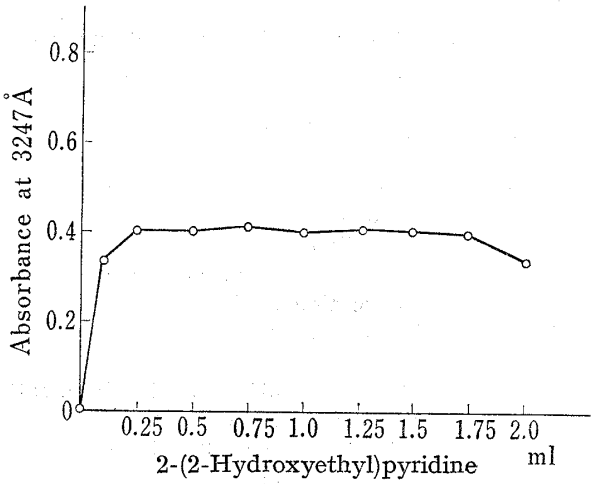

Fig. 2. Effect of the Concentration of 2-(2-hydroxyethyl)pyridine

flufenamic acid: 400 μg
0.1% $CuSO_4$ solution: 9 ml
recorder readout scale: X1
meter response: X1

(2) Amount of 2-(2-Hydroxyethyl)pyridine: To a mixture of 9 ml of 0.1% $CuSO_4$ solution and 4 ml of 100 μg/ml flufenamic acid solution (0.001N NaOH solution), 0 to 2.0 ml of 2-(2-hydroxyethyl)pyridine was added and the mixture was treated in the same way as in (1) and absorbance of the extract solution was

11) Y. Kidani, K. Inagaki, and H. Koike, "Abstr. Papers Annu. Meet. Anal. Soc. Japan," 1971, p. B236.
12) T. Sakaguchi and K. Ueno, "Metal Chelates, II," Nankodo, Tokyo, 1966 p. 134 and 154.

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measured. As shown in Fig. 2, absorbance was constant with between 0.25 and 1.75 ml of 2-(2-hydroxyethyl)pyridine, and 1.5 ml was used in the following experiments.

Conditions for Measuring Atomic Absorption——Before starting the experiment, wave length for measuring copper as an element, action current of a hollow cathode lamp, and gas flow were set up. Effect of changes in air flow on sensitivity was studied. As the maximum sensitivity was obtained with 13 to 15 liter/min of air flow, 14 liter/min was used. When the electric current of a hollow cathode lamp was varied between 10 and 30 mA, no changes in absorption took place. Therefore 15 mA was used considering the life of a lamp. When the height of a burner was changed between 0 and 5, the maximum sensitivity was obtained at 0, and measurements were conducted at 0.

From these results, a condition of measurements were set up as shown in Table II.

Table II. Analytical Conditions of Atomic Absorption Spectroscopy

Element and wavelength	: Cu, 3248 Å
Slit	: 4 (1 mm, 7Å)
Hollow cathode lamp current	: 15 mA
Fuel flow rate (acetylene)	: 2.2 l/min (0.56 kg/cm^2)
Oxidant flow rate (air)	: 14.0 l/min (1.9 kg/cm^2)
Position of burner	: 0
Recorder readout scale	: x3,[a] xl[b]
Meter response	: x2,[a] xl[b]
Chart speed	: 120 mm/min

a) analysis of perfusate
b) analysis of preparation

Before sample analysis, instruments were adjusted so as to show 25 to 35% absorption on a chart (VD-10002-A) using 5.0 μg/ml standard flufenamic acid solution (propyl acetate).

Application and Result

Application to Flufenamic acid Perfusion Experiment Using Rat Small Intestine

(1) Establishment of Basic Conditions——Preparative method for perfusate, calibration curves, and recovery tests of flufenamic acid from perfusate was examined. As flufenamic acid hardly dissolves in water, a concentration of flufenamic acid in perfusate was prepared to make 200 to 600 μg/ml solution using Krebs-Ringer bicarbonate buffer (pH 7.4).[13] To adjust the volume of water, which changed during the perfusion experiment, Phenol Red was added to the perfusate.[14c] According to the analytical method described below (2), calibration curves were drawn, and the results shown in Fig. 3(a) was obtained. When the final concentration of the working solution (propyl acetate) was made to 2.5—10 μg/ml, absorbance was measured with a scale expansion"x3", as the error became larger when measuring with scale expansion "x1". The absorbance obtained was calculated to 1/3,[15] and the absorbance is shown in Fig. 3(a), but compared with the calibration curves shown in Fig. 3(b) for flufenamic acid, which was measured with scale expansion "x1" according to the analytical method described in pharmaceutical preparation section, the sensitivity of Fig. (3a) was about 1/2 of it in the same concentration. It is assumed that the components in perfusate were affected on forming the complex, but this problem in now being examined. After the Krebs-Ringer

13) H. Wada(ed), "Ikagakuzikken Koza," Vol. 2A. Metabolism and Enzyme, Nakayama shoten, 1971, p. 286.
14) *a*) T. Nadai, R. Kondo, and A. Tatematsu, *Chem. Pharm. Bull.* (Tokyo), **20**, 1139 (1972); *b*) K. Kakemi, A. Arita, H. Sezaki, and I. Sugimoto, *Yakugaku Zasshi*, **84**, 1210 (1964); *c*) A. Tatematsu, N. Nadai, K. Sakai, and T. Sato, *Nippon Koku Eisei Gakkai Zasshi*, **19**, 509 (1970).
15) More correctly, a flufenamic acid-complex-propyl acetate solution which showed absorption value of about 10% on the measuring scale expansion "×1", was prepared and the absorption on scale expansion "×3" was calculated from the measured value which was obtained from measurement with scale expansion "×3". In this experiment, the computation of the apparatus used was about 2.85.

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bicarbonate buffer containing only Phenol Red was perfused through rat small intestine mentioned in (2) for 75 min, flufenamic acid was added to the same buffer in 200, 400 or 600 μg/ml concentration and recovery test was examined according to method (2). The coefficent of variations was 4.2, 4.5, or 2.3% (n=10), respectively, which was fairly satisfactory.

(2) **Perfusion Experiment, Analysis of Flufenamic Acid in Perfusate and Its Absorption Rate**——Male rats of wistar strain, weighing 200 to 220 g, were anesthetized with urethan and a small intestinal perfusion experiment was conducted with various concentrations of flufenamic acid according to the usual method,[13a-c] using 50 ml of the perfusate and 15 min after starting perfusion was set as time 0. After 15, 30, 45, or 60 min, two samples of 0.5 ml perfusate were taken. One sample was used for flufenamic acid determination and the other was used for adjusting the volume of water which varied during experiments.

Analysis of flufenamic acid in the perfusate was conducted as follows. To adjust the alkalinity of perfusate to *ca.* pH 10, 0.1N NaOH solution was added to 0.5 ml of perfusate and 10.5 ml of copper-pyridine-ethanol reagents was added to it. After adding accurately 20.0 ml of propyl acetate, the mixture was shaken well for 10 min and centrifuged at 4000 rpm for 10 min. (When the concentration of flufenamic acid in the initial perfusate was 200 μg/ml, this solution was used for the determination. When the concentration in the initial perfusate was either 400 or 600 μg/ml, this solution was diluted further as will be mentioned below). To 5 ml of this solution, propyl acetate was added to make 10 ml and atomic absorption was measured under the condition shown in Table II. Results obtained are summarized in Table III. Absorption rate of flufenamic acid increased with the concentration of flufenamic acid.

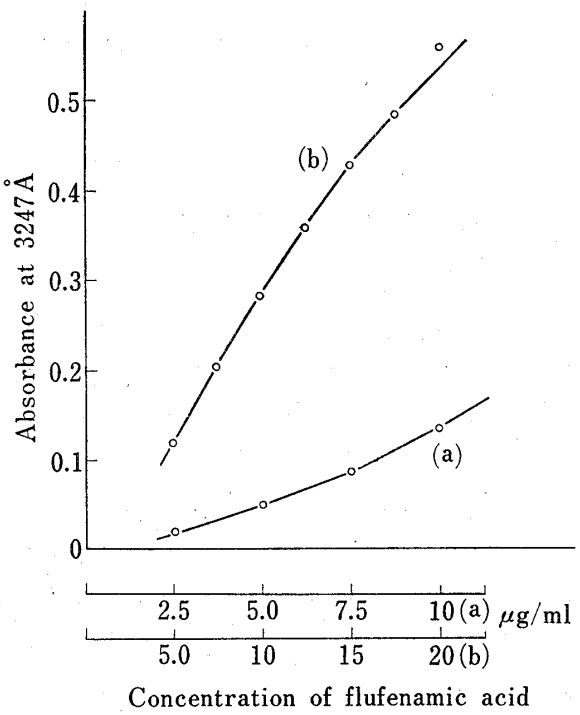

Fig. 3. Calibration Curves for Perfusate (a) and Preparation (b) of Flufenamic Acid
Atomic absorption conditions are the same as in Table II.

After the solution which was perfused at each time was acidified with HCl, flufenamic acid was extracted with $CHCl_3$ and the extract was dehydrated over Na_2SO_4. Flufenamic acid was assayed by gas chromatographic determination described in the componental analysis. Although the number of experiments was a small, the results obtained were almost the same as that from atomic absorption method.

Table III. Absorption of Flufenamic Acid from Rat Small Intestine

Concentration (μg/ml)	Absorption rate (%)[a] Mean±S.D.[b]
200	68.5±9.4
400	77.7±7.7
600	80.8±3.4

a) % absorbed in 60 min.
b) S.D.: standard deviation (n=10)

Application of Preparations Containing Flufenamic Acid

(1) **Effect of Co-existing Drugs**——According to the method described in the following (2) and used for prepared samples and for commercial preparations already marketed, a standard curve and the effect of antifebriles, which are usually administered together with flufenamic acid and anodynes in Table IV, and their vehicles were investigated: the residue which is not soluble in 0.1N NaOH solution was filtered and the supernatant was used for this test.

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Table IV. Effects of Various Ingredients on the Determination

No.	Compounds	Coexisting weight ratio	Found (%)
1	Acetylsalicylic acid	1 : 2	113.2
2	Sasapirin	1 : 2	105.5
3	Carbinoxamine maleate	1 : 5	104.9
4	Salicylamide	1 : 5	104.4
5	Carbetapentane citrate	1 : 1	102.9
6	Diphenylpyraline HCl	1 : 2	102.4
7	Aminopropyrone	1 : 2	102.4
8	Asverin citrate	1 : 2	101.9
9	Allopyrabital	1 : 2	101.0
10	*dl*-Methylephedrine HCl	1 : 2	100.5
11	Migrenin	1 : 5	100.5
12	Ascorbic acid	1 : 2	100.5
13	Thiamine HCl	1 : 2	100.0
14	Tripelennamine HCl	1 : 5	100.0
15	Antipyrine	1 : 5	100.0
16	Aluminium acetylsalicylate	1 : 2	99.5
17	Trimeprazine tartrate	1 : 2	99.5
18	Diphenhydramine tannate	1 : 2	99.5
19	Diphenylpyraline-8-chloro theophyllinate	1 : 2	98.9
20	Diphenhydramine salicylate	1 : 2	98.9
21	Caffeine	1 : 2	98.9
22	Dihydrocodeine phosphate	1 : 2	98.9
23	Aminopyrine	1 : 2	98.4
24	Chlorpheniramine maleate	1 : 5	98.1
25	Phenacetine	1 : 2	97.8
26	Allylisopropylacetylurea	1 : 5	96.6
27	Riboflavine phosphate	1 : 2	95.1
28	Dextromethorphan hydrobromide	1 : 5	95.1
29	Glycerylguaiacolether	1 : 5	95.1
30	Sodium salicylate	1 : 2	95.1
31	Noscapin	1 : 5	94.2
32	Sulpyrine	1 : 2	93.4
33	Isopropylantipyrine	1 : 2	92.9
34	Barcetin	1 : 2	92.3
35	Ethoxybenzamide	1 : 2	91.8
36	Diphenhydramine HCl	1 : 2	91.8
37	Magnesium oxide	1 : 10	112.0
38	Saccharin sodium	1 : 5	111.9
39	Heavy sanalmin	1 : 5	111.9
40	Ethyl paraoxybenzoate	1 : 10	111.3
41	Magnesium carbonate	1 : 5	110.2
42	Sodium hydrogen carbonate	1 : 10	108.8
43	Avicel	1 : 10	108.8
44	Solider	1 : 5	108.0
45	Calcium hydrogen phosphate	1 : 5	107.4
46	Lactose	1 : 5	105.1
47	Sucrose	1 : 5	105.1
48	Ethylcellulose	1 : 5	105.1
49	Polymer of 1-vinyl-2-pyrrolidinone	1 : 5	104.4
50	Heavy synthetic aluminium silicate	1 : 5	104.4
51	Perfiller 101	1 : 10	104.4
52	Light sanalmin	1 : 5	103.4
53	Cornstarch	1 : 5	102.3
54	Dried aluminium hydroxide gel	1 : 5	101.7
55	Magnesium stearate	1 : 5	101.7
56	Light silicic acid anhydrous	1 : 5	101.1
57	Amylum solani	1 : 5	100.6
58	Light synthetic aluminium silicate	1 : 5	100.6

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No.	Compounds	Coexisting weight ratio	Found (%)
59	Methylcellulose TC 5	1 : 5	100.0
60	Kaolin	1 : 5	99.4
61	Talc	1 : 5	99.4
62	Acacia	1 : 5	98.3
63	Precipitated calcium carbonate	1 : 5	98.3
64	Calcium carboxymethylcellulose	1 : 5	97.7
65	Magnesium aluminosilicate hydrate	1 : 5	97.7
66	Butyl paraoxybenzoate	1 : 5	97.2
67	Rice starch	1 : 5	93.8
68	Rosin soluble in water	1 : 5	93.2

Details on the sensitivity of this method for drugs evaluation will be reported separately. In this test when the recovery of flufenamic acid was beyond the limits of 100±5.5%, according to the accuracy of the apparatus used and the another, it was understood that interference occurred. No. 1 and No. 37 to 45 in Table IV showed positive interference, while No. 32 to 36, No. 67 and 68 showed negative interference.

Regarding the removal of interfering substances the following methods were used; acetylsalicylic acid, sulpyrin and vehicles could be removed by extracting flufenamic acid from the acidic solution with ether. In the same way barbital in barcetin could be removed by extracting flufenamic acid with $CHCl_3$. Isopropylantipyrine, ethoxybezamide and diphenhydramine could be removed by extracting flufenamic acid from the alkaline solution.

(2) Analysis of Prepared Samples and Commercial Preparations——More than 10 capsules were weighed precisely and cut in order to take out the content avoiding any lost. The packing of the capsules was weighed precisely and the weight of the content was calculated by differences before and after removing the content. An amount corresponding to about 50 mg of flufenamic acid was weighed precisely according to description and dissolved in 0.1N NaOH solution to make up to 500 ml. Two ml of this solution was pipetted to a centrifuge tube with a stopper, to which 10.5 ml of copper–pyridine–ethanol reagent were added and shaken, then 20 ml of propyl acetate were added to above mixture, shaken for 5 min and centrifuged at 4000 rpm for 5 min. The propyl acetate layer was filtered using Toyo Filter Paper No. 7, thus giving the sample solution.

To a centrifuge tube with a stopper, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, or 4.0 ml of 100 μg/ml 0.1N NaOH solution of flufenamic acid and 2.0 ml of 0.1N NaOH solution were pipetted respectively, 10.5 ml of copper–pyridine–ethanol reagent were added and treated in the same way as for the sample solutions in order to obtain standard curve and a blank (containing 2.0 ml of 0.1N NaOH solution).

Table V. Determination of Flufenamic Acid in Synthetic Preparation (I) and Commercial Sample (II)

Compounds		in 1 Capsule (mg)
Flufenamic acid		100
Cornstarch		74
Heavy synthetic aluminium silicate		20
Talc		6
(I)	$\bar{X}$ (%)	101.6
	n	10
	σ (%)	3.3
(II)	$\bar{X}$ (%)	103.5
	n	10
	σ (%)	5.5

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A standard curve was obtained by measuring the atomic absorption of the above solution in the conditions shown in Table II and the content of the capsules was calculated as shown in Table V.

Analysis of Composition of the Chelate Compound

Composition of the chelate compound mentioned above was carried out. To 20.0 ml of a propyl acetate solution containing the chelate compound, 20.0 ml of 0.1N H_2SO_4 was added, shaken for 10 min, and centrifuged at 4000 rpm for 10 min. Using the H_2SO_4 layer, the amount of Cu was measured first by atomic absorption (the standard additive method was used), and by a colorimetric method using bathocuproine sodium sulfonate.[16] prior to this experiment, it was confirmed that there was no Cu in the propyl acetate layer after shaking it with 0.1N H_2SO_4.

Flufenamic acid was analyzed either by measuring the ultraviolet absorption of the propyl acetate soltion after shaking it with 0.1N H_2SO_4 at 346 mμ[17] or by gas chromatography of the sample obtained by the addition of 0.5 ml of 0.1% santonin–$CHCl_3$ solution and 0.1 ml of N,O-bis(trimethylsilyl)acetamide after evaporation *in vacuo* of 20.0 ml of the propyl acetate solution.

As shown in Table VI, it was presumed that flufenamic acid and Cu are combined in the ratio of 1 to 1. Further to study 2-(2-hydroxyethyl)pyridine in a complex compound, propyl acetate in the upper layer was evaporated *in vacuo*, but only the oil substance was obtained. To assay this, about three mg of 2-(2-hydroxyethyl)pyridine were first added to 2.0 ml of biphenyl–$CHCl_3$ solution and 0.4 ml of N,O-bis(trimethylsilyl)acetamide, shaken for 10 min, and dissolved; 1.0 μl of this solution was injected into a gas chromatograph. This solution was assayed, but the results were not interpretable. As the 1:1 ratio of flufenamic acid and Cu had been studied in No. 1—2 of Table VI, the same ratio of flufenamic acid (dissolved in 0.1N NaOH solution) and Cu was mixed and dissolved, and in this solution, 1M 2-(2-hydroxyethyl)pyridine was added in the same ratio and mixed up. A blue crystallization was obtained in a way similar to the anthranilic acid-chelate. This crystalline compound was filtered and then recrystallized with a mixture of $CHCl_3$ and petroleum ether, and dried until it reached a constant weight at 60° *in vacuo*. It was assayed according to the method shown in Table VI No. 3. From this results, we assumed that the ratio of our chelate compound was 1:1:1. This compound was then for its elementary composition assayed. The results conformed

Table VI. Analysis of Components in Flufenamic Acid Complex

No.	Components	Analytical method[a]	Found		Mol ratio
			$\times 10^{-8}$M	%	
1	Flufenamic acid	A	7.68		1.04
	Cu	B	7.35		1.00
2	Flufenamic acid	C	6.51		1.00
	Cu	D	6.49		1.00
3	Flufenamic acid	A		59.26	1.05
	Cu	D		12.99	1.02
	2-(2-Hydroxyethyl)pyridine	C		24.49	1.00

a) A: ultraviolet absorption spectroscopy
B: atomic absorption spectroscopy
C: gas chromatography
D: visible absorption spectroscopy

16) Dotite Reagents 9th, "Dojin Co., Ltd. Research Lab," 1970, p. 41.
17) Flufenamic acid has a strong absorption at 289 mμ, but as the reagent was interfering at this wave length, absorption at 346 mμ was used.
18) T. Minamikawa and N. Yamagishi, unpublished in detail.

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the ratio 1: 1: 1. *Anal.* Calcd. for $C_{21}H_{16}O_3N_2CuF_3$: C, 54.25; H, 3.46; N, 6.02: Found: C, 54.28; H, 3.15; N, 5.94. (mp, the crystallization moistened at 199.5°, and decomposed at 200.5°). After mixing equal amount of flufenamic acid and Cu to get a constant ratio of 1: 1, the mole ratio of 2-(2-hydroxyethyl)pyridine was changed to 1—10 M according to the way rised to obtain the crystallization carried out for the elementary analysis; it was then recrystallized and dried as described above. The mp, IR and elementary analysis of each crystallization product were examined, but the ratio of flufenamic acid, Cu and 2-(2-hydroxyethyl)pyridine were 1: 1: 1 without regard to the mole ratio of 2-(2-hydroxyethyl)pyridine.

Acknowledgement The authors wish to thank Laboratory Chief, Toshio Shibazaki of the National Institute of Hygienic Sciences for his instruction and encouragement and President, Yasuo Ito and Factory manager, Juro Matsumura for having us the opportunity of publishing this paper.

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