Determination of Antipyrine in Combinations with Other Drugs

By JOSEPH LEVINE and JOHN D. WEBER

Antipyrine in pharmaceutical preparations is determined by isolation from an ether solution on a Celite–ferric chloride partition column. It is eluted free of ferric ion with chloroform and its concentration in the eluate is measured spectrophotometrically. None of the components of the usual drug combinations interfere. With slight modification, the concurrent determination of components such as caffeine, benzocaine, salicylic acid, and iodopyrine, can be made.

THE PUBLISHED procedures for the determination of antipyrine are, in general, inapplicable in the presence of the other drugs with which it is frequently formulated. These methods include precipitation with picric acid (1) or sodium tetraphenylborate (2, 3), bromination (4–6), iodination (7–9), nonaqueous titration with perchloric acid (10, 11), colorimetry (12, 13), and complexiметry (14, 15).

Methods for the determination of some of the drugs commonly combined with antipyrine may involve the same problems of isolation that are encountered in the determination of the antipyrine itself and therefore will be considered here. In the A.O.A.C. method for antipyrine and caffeine (8) the antipyrine is converted to iodopyrine, which is weighed together with the caffeine. The iodine content of the iodopyrine, determined gravimetrically as silver iodide, is a measure of the antipyrine content of the sample; the caffeine content is calculated by difference. In the method of Bister and Wolff (16) a chloroform-petroleum ether solution of antipyrine and caffeine is passed over a chromatographic column of activated alumina upon which ferric chloride is adsorbed. The caffeine passes through, while the antipyrine is retained; the latter then is eluted by methanol. After an intermediate step to remove ferric chloride, which is present in each fraction, the individual components are determined by iodometric titration. Sjöström and Nykänen (17) have reported a procedure in which an aqueous solution of the mixture is passed through a column of a strong cation exchange resin in the ferric form. The caffeine passes through, while the antipyrine is retained. No provision is made for the recovery of the antipyrine. These procedures are designed for mixtures containing approximately equal proportions of antipyrine and caffeine and are inapplicable to mixtures having a very low caffeine content.

Column partition chromatography is effective in the separation and isolation of ionizable compounds. During the flow over a column, an ionizable substance dissolved in the organic mobile phase will react with a suitable aqueous ionic immobile phase to form its water-soluble salt, which remains trapped in the immobile phase. For example, acetylsalicylic acid will be trapped by sodium bicarbonate. Caffeine will be extracted from an ether solution but not from a chloroform solution by a 4 *N* sulfuric acid trap column. Ionic trapping is not restricted to acid–base interaction. Ferric chloride solution can be used as the immobile phase to trap compounds having a phenolic hydroxyl group, thus permitting the separation of salicylic acid from the nonphenolic acetylsalicylic acid (18).

The trapped components may be recovered in several ways. Caffeine is eluted from the 4 *N* sulfuric acid with chloroform; acetylsalicylic acid is liberated from the sodium bicarbonate by acidification of the latter immobile phase *in situ;* and salicylic acid is liberated from the ferric chloride by dissociation of the complex with acetic acid.

The principles underlying the partition separations described are applicable to the isolation of antipyrine from its dosage forms, and its separation from other therapeutic substances with which it is combined in commercial formulations. On passage of an ether solution of antipyrine over a ferric chloride partition column, the antipyrine is trapped as its red complex. Pure antipyrine, free of ferric chloride, is recovered from the column simply by elution with chloroform. Although several of the previously reported procedures cited above also are based upon complexation with ferric ion, they do not provide for the facile recovery of pure antipyrine from the dissociated complex.

METHOD

Preparation of Sample

Liquid Preparations.—Transfer to a volumetric flask a quantity of sample sufficient to provide after dilution a concentration of about 0.5 mg. antipyrine per milliliter and adjust to volume with water.

Tablets.—Transfer to a volumetric flask a quantity of finely powdered sample to provide a solution of 0.5 mg./ml. Add chloroform, shake to dissolve soluble components, and adjust to volume with chloroform.

Chromatographic Columns

Use a 25 × 250 mm. test tube to which is attached a 50-mm. length of 6- to 8-mm. tubing. The tamping rod is a disk of stainless steel, aluminum, or glass, with a diameter 1 mm. less than that of the column and attached to a rod 12 to 18 in. long. Pack fine glass wool[1] in the base of the column as support.

Column A.—*Lower Stage.*—Mix 2 Gm. of Celite[2] with 1 ml. of 1 *N* NaOH to form a uniform fluffy mixture. Transfer to the column and tamp to a uniform mass using gentle pressure.

Middle Stage.—(For samples containing bases or their salts.) In a like manner, mix 3 Gm. of Celite with 2 ml. of McIlvaine pH 4.0 buffer. Transfer to column directly above lower stage.

Upper Stage.—(Use for aqueous sample preparations only.) Mix 3 Gm. of Celite with a 2-ml. aliquot of the sample preparation and transfer to

Received October 5, 1964, from the General Methods Branch, Division of Pharmaceutical Chemistry, Bureau of Scientific Research, Food and Drug Administration, U. S. Department of Health, Education, and Welfare, Washington, D. C.

Accepted for publication December 23, 1964.

[1] Pyrex Filtering Fibre, Corning Glass catalog No. 3950.

[2] Acid-Washed Celite 545, Johns-Manville Corp.

column. Dry-wash the beaker with approximately 1 Gm. of Celite to insure quantitative transfer, and tamp as above.

Column B.—Mix 4 Gm. of Celite with 3 ml. of 9% $FeCl_3$ solution. Place a small loose plug of glass wool above packing.

Determination of Antipyrine

Procedure.—(Use water saturated solvents throughout.) Mount column *A* directly above column *B*. For samples of aqueous preparations, pass three 25-ml. portions of ether over the columns; for preparations in chloroform, dilute a 2.0-ml. aliquot of the sample preparation with 25 ml. of ether, and pass it over the column, followed by three 25-ml. portions of ether. (Reserve the ether eluate for determination of accompanying constituents.) Remove column *A*.

Place as a receiver under column *B* a 100-ml. volumetric flask containing 10 ml. of methanol and elute to volume with chloroform. Determine the absorbance at the maximum at about 272 mμ and compare with that of a standard solution, prepared by adding 10 ml. of ether to a solution of 1 mg. of pure antipyrine in 10 ml. of methanol and diluting to 100 ml. with chloroform.

Determination of Other Constituents

With minor modifications, the above procedure can be adapted to the concurrent determination of many of the components with which antipyrine is formulated.

Benzocaine.—Add 50 ml. of alcohol to the ether eluate from column *B* and concentrate to approximately 25 ml. (Further concentration will result in loss of benzocaine by evaporation.) Transfer to a 100-ml. volumetric flask and dilute to volume with alcohol, adjusting the concentration by further dilution if necessary. Determine the absorbance at 293 mμ and compare it with that of a standard solution of benzocaine.

Salicylic Acid.—After elution of antipyrine from column *A* with ether, place a 100-ml. volumetric flask as receiver under column *A*. Elute salicylic acid with 1.0 ml. of acetic acid in 10 ml. of chloroform and complete the elution with a solution of acetic acid in chloroform (1 in 100) to make to volume (19). Determine the absorbance of this solution and compare with that of a standard solution of salicylic acid at the maximum at about 310 mμ.

Caffeine.—Place column *C* (3 Gm. of Celite plus 2 ml. of 4 *N* H_2SO_4) below column *B*. After elution with ether, separate the columns and place a 50-ml. volumetric flask as receiver under column *C*. Elute the caffeine with chloroform to volume (19). Determine the absorbance at the maximum at about 276 mμ and compare with that of a standard solution of caffeine.

If the formulation contains a small proportion of caffeine, the quantity of sample required to furnish sufficient caffeine for spectrophotometric measurement will necessarily contain a larger quantity of antipyrine than the amount specified above. The additional quantity is accommodated readily by the columns; the antipyrine eluate from column *B* simply must be diluted to a concentration suitable for measurement.

Iodopyrine in Formulation Containing Caffeine.—Replace column *B* with one containing 5 Gm. of Celite plus 3 ml. of 9% $FeCl_3$. (The relatively larger volume of ether required to elute iodopyrine will move the antipyrine down column *B* to a significant degree, evidenced by the position of the red antipyrine–$FeCl_3$ band. The longer $FeCl_3$ layer prevents the movement of this band to the bottom of the column.) Dissolve a quantity of sample in chloroform to provide 0.25 mg. of caffeine per milliliter. Prewash the columns with 150 ml. of ether. Pass over the columns a 2-ml. aliquot of the sample preparation diluted with 25 ml. of ether, followed by five 25-ml. portions of ether. Evaporate the eluate to dryness. Dissolve the residue in 25.0 ml. of methanol and determine the absorbance at the maximum at about 275 mμ. Compare with absorbance of a standard solution of iodopyrine.[3]

TABLE I.—RECOVERIES OF STANDARD PREPARATIONS[a]

Sample	Substance Detd.	Recovery, %
Antipyrine N.F.	Antipyrine	100.2
		100.0
		99.2
Ear drops	Antipyrine	99.8
		99.8
		98.5
	Benzocaine	100.8
		98.7
		98.5
Nasal spray	Antipyrine	99.7
		99.7
		99.7
Dichloralphenazone	Antipyrine	100.7
		99.8
		100.3
Antipyrine–salicylic acid	Antipyrine	99.5
		99.9
	Salicylic acid	100.0
		99.4
Antipyrine–caffeine	Antipyrine	99.1
		99.0
	Caffeine	99.3
		99.3
Antipyrine–caffeine–iodopyrine	Antipyrine	101.0
	Caffeine	97.4
		95.8
	Iodopyrine	93.0
		94.8

[a] Dosage forms prepared to simulate the commercial preparations reported in Table II.

RESULTS AND DISCUSSION

This procedure has been applied to the determination of antipyrine in tablets, ear drops, and nasal sprays, including preparations containing benzocaine, caffeine, salicylic acid, iodopyrine, 8-hydroxyquinoline, acetaminophen, pyrilamine maleate, isometheptene mucate, and chloral hydrate (present as dichloralphenazone, a crystalline addition complex of antipyrine and chloral hydrate).

Recoveries of antipyrine and of the added components in simulated commercial products are presented in Table I. Analyses of commercial products are given in Table II. The analyses of two of the commercial samples reported in Table II differed substantially from their label declarations. The

[3] Iodopyrine was prepared by method of Bougault (7) and recrystallized by dissolving in chloroform and precipitating with ether; needles, m.p. 160.5°–160.8°.

TABLE II.—ANALYSES OF COMMERCIAL PRODUCTS

Sample	Composition	Found	Label Claim, %
Ear drops	Antipyrine, 54.0 mg./ml.; benzocaine, 14.0 mg./ml.; 8-hydroxyquinoline, glycerol	Antipyrine	104.4, 103.7
		Benzocaine	102.1, 102.8
Nasal spray	Antipyrine, 0.28%; D-desoxyephedrine, methyldodecyl benzyl trimethyl-ammonium chloride	Antipyrine	98.2 97.1 97.1
Throat spray	Antipyrine, 0.30%; pyrilamine maleate, methyl salicylate, menthol, aromatics	Antipyrine	98.7 99.7
Antipyrine–salicylic acid tablets	Antipyrine, 4.3 gr.; salicylic acid, 3.2 gr.	Antipyrine	92.5 92.5 92.3
		Salicylic acid	89.3 89.1 89.1
Tablets	Dichloralphenazone, 100 mg.[a]; isometheptene mucate	Antipyrine	102.6 101.9 103.2
Tablets	Antipyrine, 435 mg.; citrated caffeine, 50 mg.[b]; iodopyrine, 15 mg.	Antipyrine	100.2 100.2
		Caffeine	177.6 179.0
		Iodopyrine	99.0 97.9

[a] Equivalent to 36.26 mg. of antipyrine and 63.74 mg. of chloral hydrate. [b] Equivalent to 25 mg. of caffeine.

precision of these analyses, the quantitative recoveries obtained with standard simulated samples (Table I)—and in the case of the high caffeine value—the purity of the isolated material, establish the validity of the analyses reported.

Applicability of the procedure is not restricted to the compounds, nor the combinations reported. The products analyzed in the current study were those listed in such standard reference publications as the "Physicians' Desk Reference" and the "American Drug Index." Undoubtedly, many other combinations are marketed. While the procedure described may not be applicable to all conceivable combinations, it can serve as a guideline for an approach to any given formulation.

REFERENCES

(1) Lemaire, A., *Analyst*, **30**, 22(1905).
(2) Schultz, O., and Mayer, G., *Deut. Apoth. Ztg.*, **92**, 358 (1952).
(3) Bobtelsky, M., and Cohen, M., *Anal. Chim. Acta*, **22**, 328(1960).
(4) Medis, V., *Österr. Chem. Ztg.*, **42**, 290(1939).
(5) Schulek, E., and Kovacs, J., *Ber. Ungar. Pharm. Ges.* **16**, 344(1940); through *Chem. Abstr.*, **34**, 7063(1940).
(6) Hofstetter, R., and Muhlemann, H., *Pharm. Acta Helv.*, **38**, 218(1963).
(7) Bougault, J., *J. Pharm. Chim.*, **15**, 337(1917).
(8) "Official Methods of Analysis," 9th ed., Association of Official Agricultural Chemists, Washington, D. C., 1960, Sec. 32.144–32.145, p. 492.
(9) Gengrinovich, A., *Farm. Zhur.*, **13**, 15(1940); through *Chem. Abstr.*, **35**, 3559(1941).
(10) Ellert, H., Jasinski, T., and Pawelczak, I., *Acta Polon. Pharm.*, **16**, 235(1959); through *Chem. Abstr.*, **54**, 1182 (1960).
(11) Rink, M., Lux, R., and Reimhofer, M., *Pharm. Ztg.*, **104**, 1380(1959); through *Chem. Abstr.*, **54**, 13550(1960).
(12) Celechovsky, J., and Svobodova, D., *Cesk. Farm.*, **8**, 380(1959); through *Chem. Abstr.*, **54**, 5015(1960).
(13) Nambara, T., *Yakugaku Zasshi*, **80**, 460(1960); through *Chem. Abstr.*, **54**, 17796(1960).
(14) Bobtelsky, M., and Cohen, M., *Anal. Chim. Acta*, **22**, 270(1960); through *Chem. Abstr.*, **54**, 11383(1960).
(15) Dick, J., and Ristici, J., *Acad. Rep. Populare Romine, Baza Cercetari Stiint. Timisoara, Studii Cercetari Stiinte Chim.*, **9**, No. 3–4, 269(1962); through *Chem. Abstr.*, **59**, 1095 (1963).
(16) Bister, F., and Wolff, J., *Z. Anal. Chem.*, **137**, 327 (1953).
(17) Sjöström, E., and Nykänen, L., THIS JOURNAL, **47**, 248(1958).
(18) Levine, J., *ibid.*, **50**, 506(1961).
(19) *Ibid.*, **46**, 687(1957).