catheterization. As shown in Table II, positive tests were obtained with the PVA-iodine reagent.

By a series of experiments which followed, rabbits were fed boric acid solutions of various concentrations (as shown in Table II) *via* a stomach tube. Samples of urine were collected by catheterization and tested for boron content. In all instances, control samples were collected from untreated animals. The results from these tests are shown in Table II.

DISCUSSION

As can be seen from Table I, as little as 0.3 mg. of H_3BO_3 (0.05 mg. B) can be detected in both aqueous and urine solutions with the PVA-borate-iodine reaction. The constituents normally found in urine did not interfere with the test, and quantities of boric acid above 0.3 mg. could easily be detected.

Starch, as an environmental contaminant, will give a positive test; therefore, its presence, if suspected, must be ruled out as suggested in a previous paper by Monte-Bovi and Sciarra (3).

The results of the PVA-iodine test when applied to samples of urine collected from animals treated or fed boric acid indicate the suitability of this test for these purposes. While we did not repeat these experiments on humans for obvious reasons, others (17, 19) have cited that boron compounds when absorbed by humans are found in body fluids and tissues.

While there are several other tests available for the detection of small quantities of boric acid as cited in the literature, the PVA-borate-iodine complex offers another accurate and sensitive method. This test can also be used as a confirmatory test when other methods give inconclusive results.

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Chromatographic Method for the Simultaneous Determination of Aspirin, Caffeine, and Acetaminophen

By K. THOMAS KOSHY

A procedure for the quantitative determination of aspirin, caffeine, and acetaminophen in a mixture is described using a modified chromatographic technique developed by Levine. In addition, this procedure can be used for the estimation of salicylic acid, if present in the mixture.

A REMARKABLE chromatographic technique for the analysis of mixtures of aspirin, caffeine, and phenacetin was developed by Levine (1) using a duplex column having aqueous solutions of sodium bicarbonate and sulfuric acid as immobile phases on a Celite support. The technique was extended by Heuermann and Levine (2) to the analysis of more complex mixtures. In principle, this technique is adaptable to the separation of acidic, basic, and neutral components in a mixture, provided suitable solvents are available. Since there are many formulations containing aspirin, caffeine, and acetaminophen (APAP), the purpose of this study was to determine these constituents in such a mixture. The study was further extended to the determination of *p*-aminophenol and salicylic acid in the presence of these components. The latter two are hydrolytic degradation products of APAP and aspirin, respectively.

EXPERIMENTAL

Preparation of Chromatographic Column.—Commercial acid-washed kieselguhr (Celite 545 Johns-Manville Corp.) was used in this study. It contained impurities which interfered in the analysis of *p*-aminophenol and was purified by boiling for 1 hour with concentrated hydrochloric acid, washing with water, and drying at 100°. The column was prepared as described by Heuermann and Levine (2). However, column arrangement was reversed. The column containing sulfuric acid as the immobile phase (column A) was mounted in such a manner that the effluent would flow into the column containing sodium bicarbonate as the immobile phase (column B). This was done to avoid contact of *p*-aminophenol with the sodium bicarbonate column since it was observed that this compound was not stable in the presence of bases.

Procedure.—Water-washed solvents were used throughout the chromatographic separation of the constituents. The sample size could be varied within wide limits depending on the composition of the material to be analyzed. Ethyl acetate was used as solvent for the sample. Chloroform could be used, provided the sample was first dissolved in a few milliliters of alcohol. However, excess alcohol should be avoided, as this would remove water from the column.

The columns mounted as described above were washed with 50 ml. of ethyl ether. The accurately

Received January 31, 1964, from the Corporate Pharmacy Research Laboratory, Miles Laboratories, Inc., Elkhart, Ind.
Accepted for publication March 19, 1964.
The author thanks Lester L. Shankle for technical assistance in this study.

TABLE I.—ANALYSIS OF KNOWN MIXTURES OF APAP, ASPIRIN, CAFFEINE, AND *p*-AMINOPHENOL HYDROCHLORIDE

	Acetaminophen			Aspirin			Caffeine			*p*-Aminophenol HCl		
	Added, mg.	Found, mg.	Recovery, %	Added, mg.	Found, mg.	Recovery, %	Added, mg.	Found, mg.	Recovery, %	Added, mg.	Found, mg.	Recovery, %
1	491	491	100.0	977	960	98.3	103	103	100	...	...	...
2	330	325	98.5	647	627	96.9	64.3	65.9	102.5	...	...	...
3	650	657	101.1	1300	1303	100.2	137	136	99.3	...	...	...
4	500	512	102.4	507	499	98.4	104	103	99.0	...	...	...
5	511	500	97.9	999	981	98.2	162	159	98.2	...	...	...
6	503	506	100.6	1000	983	98.3	121	121	100.0	...	...	...
7	125	124	99.2	220.5	218	98.9	29.8	30.1	101.0	1.9	2.2	115.8
		125	100.0		215	97.5		30.1	101.0		2.0	105.3
		123	98.4		216	98.0		30.1	101.0		2.1	110.5
8	125	126	100.8	220.5	218	98.9	29.8	29.8	100.0	0.97	1.1	113.4
		127	101.6		218	98.9		30.1	101.0		1.2	123.7
		126	100.8		219	99.3		30.1	101.0		1.1	113.4
9	125	127	101.6	220.5	223	101.1	29.8	30.1	101.0	3.9	3.9	100.0
		127	101.6		222	100.7		30.1	101.0		3.9	100.0
		127	101.6		223	101.1		30.0	100.7		3.8	97.4

TABLE II.—ANALYSIS OF TWO COMMERCIAL TABLET FORMULATIONS OF APAP, ASPIRIN, AND CAFFEINE

	Acetaminophen			Aspirin			Caffeine		
	Labeled Amount, mg.	Found, mg.	Labeled Amount, %	Labeled Amount, mg.	Found, mg.	Labeled Amount, %	Labeled Amount, mg.	Found, mg.	Labeled Amount, %
1	125	120.4	96.3	230	229.8	99.9	30	29.0	96.7
		121.0	96.8		231.8	100.8		29.0	96.7
		119.3	95.4		230.5	100.2		29.0	96.7
2	162.5	165.0	101.5	325	314.0	96.6	32.5	32.5	100.0
		166.0	102.2		317.0	97.5		33.3	102.5
		162.0	99.7		317.0	97.5		33.5	103.1

weighed sample powder was dissolved in 100 ml. ethyl acetate in a volumetric flask. Quick dissolution was effected if the flask was heated gently on a steam bath for a few minutes. A suitable aliquot (2–5 ml.) was pipeted onto column A and allowed to run through column B into a 100-ml. volumetric flask. After all the solution had passed onto the adsorbent, the columns were washed with two 10-ml. portions of ether, allowing the first portion to run through the column before adding the second. More ethyl ether was passed through the columns until about 90 ml. was collected. The flask was removed and ether added to volume. This fraction contained APAP. A suitable aliquot was evaporated to dryness on a steam bath. The residue was dissolved in ethanol and diluted to a definite volume.

A second 100-ml. volumetric flask was placed as the receiver and the columns eluted with about 50–60 ml. of chloroform and brought to volume with more chloroform. This fraction contained caffeine. If necessary, a suitable dilution was prepared using chloroform as a diluent.

Column A was now removed but not discarded. Column B was eluted with 5 ml. of 10% glacial acetic acid in chloroform, followed by 90 ml. of 1% glacial acetic acid in chloroform. The effluent was collected in a 100-ml. volumetric flask and brought to volume with 1% glacial acetic acid in chloroform. This fraction contained aspirin and any salicylic acid that was present in the sample. Suitable dilutions, if necessary, were prepared with chloroform.

Column A containing *p*-aminophenol was eluted with approximately 40 ml. of ethyl ether to remove the chloroform from the previous operation. The ether washings were discarded. The Celite support was extruded from the column with air under pressure and collected in a 150-ml. beaker. The ether was evaporated by gentle heating on a steam bath. Exactly 20 ml. of 0.1 *N* HCl was added to the Celite, stirred to dissolve the *p*-aminophenol, and filtered through Whatman No. 44 filter paper. A 5-ml. aliquot of the filtate was used for the colorimetric determination of *p*-aminophenol using 1-naphthol as described by Greenberg and Lester (3).

The absorbance of each solution was determined on a Beckman model DU spectrophotometer against corresponding reagent blanks. The readings were taken at 245 mμ for APAP, 276 mμ for caffeine, 278 mμ for aspirin, 310 mμ for salicylic acid, and 580 mμ for *p*-aminophenol. The concentrations were calculated from the absorbance values of standard solutions of pure materials. The absorbance of aspirin is affected by acetic acid. Therefore, chloroform containing the same amount of acetic acid as the sample was used to prepare standard solutions of aspirin. This solution was freshly prepared for each analysis. In the determination of *p*-aminophenol, it was necessary to use a standard solution of *p*-aminophenol hydro-

TABLE III.—EFFECT OF COLUMN ON ABSORBANCE OF ASPIRIN SOLUTIONS

	A, 278 mμ		*A*, 310 mμ	
Aspirin, mcg./ml.	Original	Soln. Through Column	Original	Soln. Through Column
0.00	0.000	0.000		
24.17	0.171	0.170	0.000	0.020
48.34	0.360	0.355	0.000	0.028
72.51	0.550	0.540	0.000	0.036
96.68	0.730	0.720	0.007	0.049

TABLE IV.—ANALYSIS OF MIXTURES OF ASPIRIN AND SALICYLIC ACID (SA)

Added, mg.		Found, mg.		Recovery, %		
Aspirin	SA	Aspirin	SA	Aspirin	SA Exptl.	SA Corrected
24.16	00.00	23.80	...	98.5	...	...
24.16	3.02	24.16	3.62	100.0	119.9	100.0
24.16	6.03	24.21	6.51	100.2	108.0	99.2
24.16	9.05	23.94	9.67	99.1	106.9	101.0
24.16	12.07	24.76	12.50	102.5	103.6	99.3

chloride which was passed through the column and treated in the same manner as the sample. This standard was prepared by completely hydrolyzing APAP to *p*-aminophenol hydrochloride by refluxing for 30 minutes a solution of 0.2 Gm. of pure APAP in 45 ml. of 8 *N* HCl. After cooling, it was diluted to exactly 100 ml. with ethanol. A second dilution of 1 to 100 was made with ethyl acetate. Five milliliters of this solution was used as standard.

As a guide to the selection of sample size, the dilution of the effluent, and the preparation of the standard solutions, the following concentrations of materials gave the indicated absorbance reading: 50 mcg./ml. aspirin, 0.360; 10 mcg./ml. caffeine, 0.485; 5 mcg./ml. APAP, 0.430; 25 mcg./ml. salicylic acid, 0.740; and 2 mcg./ml. *p*-aminophenol, 0.370 (1-cm. cells).

RESULTS AND DISCUSSION

Results obtained on the analysis of synthetic mixtures of aspirin, caffeine, APAP, and *p*-aminophenol hydrochloride are given in Table I.

Quantitative recoveries were obtained for APAP, aspirin, and caffeine. There is good precision but variability in percentage recoveries for *p*-aminophenol. It may be mentioned that the quantities of *p*-aminophenol present in the mixtures are much higher than would normally be encountered in a tablet formulation of APAP. It is significant that *p*-aminophenol can be separated from the other components of the mixture, but the analysis of *p*-aminophenol itself is only of academic interest since the allowable limit for a tablet containing 125 mg. of APAP is only 0.06 mg.

Data on the analysis of two commercial tablet formulations of APAP, aspirin, and caffeine are shown in Table II. Most tablet excipients are either insoluble in ethyl acetate or would not absorb ultraviolet light in the concentration in the final dilutions used for spectrophotometric analysis. Therefore, this procedure should be applicable to other tablet formulations containing these active ingredients.

Attempts were made during this study to make simultaneous quantitative determinations of all components of a mixture of aspirin, APAP, caffeine, *p*-aminophenol, and salicylic acid. The results were always high for salicylic acid. Yet if there was no aspirin in the mixture, the results were very satisfactory for this compound. In these experiments, aspirin was allowed to remain on the column for the minimum time that was possible to prevent hydrolysis. Freshly prepared solutions of sodium bicarbonate were used to prepare the columns, as it was known that the pH of sodium bicarbonate increases on standing. The possibility of interference from impurities from Celite was ruled out because solvents alone passed through the column did not have absorbance at 310 mμ. A mixture of APAP, caffeine, and *p*-aminophenol hydrochloride passed through the columns under identical conditions did not cause interference. Levine (1) had reported results on the analysis of two mixtures of aspirin, phenacetin, caffeine, and salicylic acid. Although results for salicylic acid were high in both cases, no comments were made by the author. A solution of aspirin in chloroform containing 1.5% acetic acid has practically no absorption at 310 mμ, the wavelength of maximum absorption for salicylic acid. However, a solution of aspirin, which has been passed through the column, shows a slight absorption at this wavelength. This is illustrated in Table III.

It appears that the interference may be the result of slight hydrolysis of aspirin or some interaction of aspirin with the column material. This phenomenon was observed in all the experiments. The absorbance at 310 mμ for the aspirin solution passed through the column would cause an appreciable percentage error in the salicylic acid analysis because of the relatively small concentration of the latter in a realistic mixture of the two substances. This seemingly explains the results obtained from mixtures of aspirin and salicylic acid as shown in Table IV. The per cent recovery of aspirin was good, that of salicylic acid was high, but decreased as the salicylic content was increased.

Table III reveals that for solutions of aspirin passed through the columns, which gave absorbance readings of 0.360 and 0.550 at 278 mμ, the corresponding readings at 310 mμ were 0.028 and 0.036. Since it is possible to adjust the concentration of aspirin in a mixture containing salicylic acid to obtain a reading of about 0.4 at 278 mμ, it would seem possible to obtain a good approximation of salicylic acid content by subtracting 0.03 from the observed absorbance at 310 mμ. This correction factor was applied, and the results obtained are shown in the last column of Table IV. As can be seen, the corrected results are within 1% of theoretical, apparently validating this correction factor. However, this value might vary slightly under different operating conditions and should be determined experimentally.

SUMMARY

Mixtures of acetaminophen, aspirin, and caffeine were separated by a chromatographic procedure and determined quantitatively by ultraviolet spectrophotometry. This method is applicable to stability samples containing hydrolytic degradation products of acetaminophen and aspirin—namely, *p*-aminophenol and salicylic acid, respectively. In addition, salicylic acid can also be determined to obtain a reasonable estimation of aspirin stability.

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