evaporated and the residue dried. The impure cantharidin was washed twice with petroleum ether (5 cc.) to remove possible traces of fatty acids. By the previously outlined method, the cantharidin residue was titrated and the amount determined.

Table XI.—Results Obtained in the Assay of Tincture of Cantharides

Sample	Gm. of Cantharidin in 100 Cc. of Tincture	Per Cent Deviation from Mean[a]
1	0.0236	− 4.83
2	0.0265	+ 6.85
3	0.0274	+10.48
4	0.0245	− 1.22
5	0.0221	−10.88

[a] Mean = 0.0248 Gm.

SUMMARY AND CONCLUSIONS

1. A number of samples of Cantharides have been assayed according to the U. S. P. XI method. It was found that most of these were below the U. S. P. standard of 0.6%. These low results were shown to be due partially to losses of cantharidin, due to its volatility, during the evaporation procedure of the assay, and partially to the method used for the removal of fat from the extracted cantharidin. It was shown that the residue, obtained in the process and weighed as cantharidin, is not all cantharidin, but contains much inert material that is difficult to separate.

2. An improvement on the U. S. P. method has been suggested and tried out. This method requires only a very short period of time for evaporation, as compared to the official method, and determines the cantharidin content of the extracted residue by titration. Although this method yields results higher than the U. S. P. requirement, it has the advantages of being less tedious and time-consuming, and overcomes the loss of cantharidin due to volatilization. It does not overcome the loss of cantharidin sustained in the removal of fat from the extracted residue.

3. In a study of the different parts of the U. S. P. method it has been shown that the solvent of benzene and petroleum ether is satisfactory for the complete extraction of cantharidin. Chloroform was also shown to extract it completely; alcohol was shown to be a poor, unsatisfactory solvent.

4. Attempts to improve the manner in which fats and inert material are removed from the extracted residue were unsuccessful. This part of the assay procedure is in need of further work.

5. Preliminary results on the application of the titration method for estimating the cantharidin content of the tincture are reported. It is recommended that this method is worthy of further investigation for the assay of both the drug and the tincture.

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The Separation and Determination of Aminopyrine in Mixtures

*By L. E. Warren**

INTRODUCTION

The use of synthetic organic substances in medicine is rapidly increasing. During the past two or three decades, the tendency to mix two or more of the synthetics in a single dose has been in evidence. Such mixtures as acetophenetidin, acetylsalicylic acid and caffeine, acetophenetidin and salol, aminopyrine and phenobarbital, aminopyrine and caffeine, and acetylsalicylic acid and phenobarbital are not uncommon. Occasionally, as many as four active medicinal ingredients are found in a single dosage form (acetophenetidin, aminopyrine, caffeine and barbital or phenobarbital).

Satisfactory methods are not available for separating aminopyrine from the mixtures in which it is found. Due to the numerous inquiries which have been received by the Food and Drug Administration, an attempt

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was made to devise methods for the separation of aminopyrine from caffeine, acetophenetidin, barbital, phenobarbital, antipyrine and cinchophen, as well as various combinations of these drugs.

HISTORICAL

Several quantitative procedures for separating aminopyrine from other drugs have been recorded. Patein (1) separated aminopyrine from antipyrine by converting the antipyrine into the insoluble diantipyrine methane, and recovered the aminopyrine (from the filtrate) by extracting with chloroform. Astruc and Pégurier (2) determined aminopyrine by precipitation of the picrate and titrating the excess picric acid. Oliveri-Mandalà and Calderaro (3) determined aminopyrine in the presence of acetylsalicylic acid and antipyrine by heating the mixture with concentrated sodium hydroxide solution in an atmosphere of hydrogen and estimating the liberated amine. Borloz (4) has reviewed and evaluated the foregoing methods for separating aminopyrine from antipyrine.

Machtou (5) determined aminopyrine by precipitation with an excess of mercuric chloride and determination of the excess mercuric salt by the Denigés potassium cyanide–silver nitrate procedure. Miko (6) attempted to separate aminopyrine from acetophenetidin and caffeine by the differences in the solubilities of the three substances in water and ether. Schulek and Menyhárth (7) determined aminopyrine in presence of antipyrine, acetanilid, acetophenetidin and caffeine by treating with 0.1*N* potassium permanganate in presence of 0.1*N* sodium hydroxide and potassium iodide. Sulfuric acid was added and the liberated iodine titrated with sodium thiosulfate. Warren (8) separated aminopyrine from phenobarbital by extracting the aminopyrine from an alkaline solution by chloroform in an automatic extractor. The solution was then made acid and the phenobarbital removed by a fresh portion of chloroform.

Van Giffen (9) separated acetophenetidin, aminopyrine, caffeine, quinine sulfate and magnesium oxide by extracting the acetophenetidin, aminopyrine, caffeine and traces of quinine sulfate with chloroform. Hot diluted sulfuric acid removed the aminopyrine and quinine sulfate from the chloroform and the quinine in this fraction was separated from the aminopyrine by its insolubility in sodium hydroxide solution. Aminopyrine was recovered from the alkaline filtrate by shaking with chloroform. Acetophenetidin and caffeine were separated by the difference in their solubilities in water and by treatment with iodine solution to precipitate caffeine.

By means of complex phosphotungstomolybdic and arsenotungstomolybdic reagents, Wachsmuth (10) colorimetrically determined aminopyrine in the presence of acetanilid, acetophenetidin, antipyrine, caffeine and quinine. Duquénois (11) reported that a solution of cadmium iodide (or potassium cadmium iodide, Marmé's reagent) gives a white precipitate with aqueous solutions of aminopyrine.

Payne (12) separated aminopyrine from phenobarbital by solution of the phenobarbital in *N* sodium hydroxide and removal of the aminopyrine by shaking with chloroform. The alkaline, aqueous solution was acidified and the phenobarbital removed by a mixture of chloroform and ether. He also tried separating the aminopyrine by combining it with sulfuric acid (1 + 9) and removing the phenobarbital with an immiscible solvent. Sinton and Rotondaro (13) separated aminopyrine from caffeine and antipyrine by shaking the mixture with chloroform in presence of 5 per cent sulfuric acid.

EXPERIMENTAL

Tests were carried out to ascertain whether or not the aminopyrine cadmium iodide complex described by Duquénois (11) was sufficiently insoluble to be of service for a quantitative determination. Samples of this complex were prepared from aminopyrine and cadmium iodide. The solubility of this material at 8–10° was found to be about 1 part in 1000 parts of water. This value was thought to be too great for an accurate quantitative procedure.

Due to some encouraging results (unpublished) for the separation of mixtures of acetylsalicylic and salol, acetophenetidin and salol, and acetanilid and salol by methods based upon their differential solubilities in organic solvents, an attempt was made

to separate aminopyrine from various mixtures by such a procedure. According to figures given in Table I, it seems likely that aminopyrine and cinchophen could be separated in this manner by the difference in their solubilities in carbon tetrachloride. Some tests were made and the results gave recoveries of 115 per cent for aminopyrine. Possibly the high results may be explained by some form of combination between aminopyrine and cinchophen, the resulting product having a different solubility in carbon tetrachloride. Since most of the mixtures under consideration in this paper contained aminopyrine and some acidic medicinal, it was thought that similar difficulties might be encountered throughout and this method was abandoned.

After numerous trials, the following method was adopted:

From 1 to 2 Gm. of the mixture were weighed into a separator, 25 cc. of approximately *N* sulfuric acid added and the mixture shaken with 40 cc. of chloroform (certain excipients do not dissolve). The extraction was repeated five times using 25-cc. portions of chloroform. Each chloroform fraction was washed successively through 10 cc. of 3.5 per cent sulfuric acid and 10 cc. of 1 per cent hydrochloric acid. The chloroform was filtered through cotton into a weighed Erlenmeyer flask. Most of the chloroform was recovered and the remainder allowed to evaporate spontaneously while the container was rotated in an inclined position. A few cc. of anhydrous ether were added and the residue again heated to dryness to remove last traces of chloroform (14). The residue was dried at 80° and weighed as anhydrous caffeine.

Table I.—Solubilities of Seven Organic Substances in Seven Solvents

(Compiled from various sources)

	(1 Gm. of Substance Soluble in Stated Number Cc. of Solvent)						
Substance	Alcohol	Benzene	Carbon Tetrachloride	Ether	Chloroform	Petroleum Benzin	Water
Acetophenetidin	15	1755	1715	130	14	10,200	1310
Aminopyrine	1.5	12	7.15	13	1	238	18
Antipyrine	1.3	14	61	43	1	3220	Less than 1
Barbital	14	2000	9100	35	75	100,000	130
Caffeine (hydrated)	66	100	700	530	5.5	20,200 17,600	46
Cinchophen	120	4771 5380	17,500	100	400	60,000	11,000
Phenobarbital	8	700	Slightly soluble	13	40	18,300	1000

Separation of Aminopyrine from Caffeine.—Preparations of aminopyrine and caffeine only do not appear to be on the market, but mixtures of these ingredients with others, such as acetophenetidin, extract of hyoscyamus and phenobarbital, are available. Since the analysis of the more complex preparations involves the separation of the aminopyrine from the caffeine, it was considered important that methods for the separation of these two substances be studied first.

In the preliminary quantitative tests, it was noted that *N* hydrochloric acid[1] did not completely hold the aminopyrine when the mixture was shaken with chloroform to remove the caffeine. After some experimentation and correspondence with other members of the Administration, sulfuric acid (1 + 9) was chosen for further study. Most of the results obtained in the earlier portion of this study were obtained with this strength of acid and are so designated in the tables. Later experiments indicated that the optimum strength of sulfuric acid was 5 per cent for the extractions and 3.7 per cent for the first washing as recommended by Sinton and Rotondaro (13). In the later trials, the author employed one further washing of the chloroform extract with one per cent hydrochloric acid.

The washings were added to the mixture in the first separator, the solution made slightly alkaline with ammonia T.S. and shaken out with 25-cc. portions of chloroform until all of the aminopyrine had been removed. Each chloroform fraction was washed through 10 cc. of water containing a few drops of ammonia T.S., the chloroform filtered through cotton and the filtrate collected in a weighed Erlenmeyer flask. The solution was evaporated and the residue treated as for caffeine in the preceding paragraph.

Known mixtures of aminopyrine and anhydrous caffeine were prepared with and without starch as a diluent. Portions of these mixtures were analyzed according to the method outlined above and the results are summarized in Table II.

Determination of Aminopyrine and Acetophenetidin in Mixtures.—Five analyses were made by the above method on an authentic mixture containing 18.43 per cent acetophenetidin and 64.0 per cent aminopyrine. The recoveries for acetophenetidin ranged from 98.4 to 101.1 per cent with an average of 100 per cent and the aminopyrine determinations ranged from 99.1 to 101.1 per cent with an average of 100.2 per cent.

Separation of Aminopyrine and Barbital.—A mixture of aminopyrine, barbital and starch in approxi-

[1] Hydrochloric acid of this concentration was selected because this is employed as a solvent in the A. O. A. C. official method for the assay of aminopyrine tablets. However, in this case the acid is neutralized before removal of the aminopyrine by chloroform.

Table II.—Analyses of Aminopyrine and Caffeine Mixtures

Number of Analyses	Acid Used	Aminopyrine			Caffeine		
		Found, %	Theory, %	Recovery, %	Found, %	Theory, %	Recovery, %
Without diluent							
3	*N* HCl	89.5	90.0	99.4	10.58	10.03	105.5
2	H_2SO_4 (1 + 9)	91.13	89.7	101.6	10.11	10.28	98.3
5	H_2SO_4 5%	87.93	88.11	99.8	12.19	11.89	102.5
With added starch							
6	H_2SO_4 5%	70.33	71.59	98.2	9.83	9.57	102.7

mately the proportions of commercial preparations was subjected to analysis by the following methods:

1. The method previously outlined for the separation of aminopyrine and caffeine was modified slightly due to the low solubility of barbital in chloroform. A mixture of chloroform and ether (2 + 1) was used and a greater number of extractions were required to completely remove the barbital.

2. The material was suspended in water, the mixture made alkaline with sodium hydroxide T.S. (to hold the barbital) and the aminopyrine extracted with small successive portions of chloroform. The chloroformic solutions were united, washed successively with water containing 1 per cent of sodium hydroxide and 1 per cent of ammonium hydroxide and evaporated. The residue was treated as previously described and weighed as aminopyrine. The alkaline solution from which the aminopyrine had been removed was then acidified with sulfuric acid T.S. and the barbital removed by shaking with chloroform. The chloroform solution was washed with water, the chloroform evaporated, the residue treated with the usual precautions and weighed as barbital.

In five analyses by the first method, the recoveries of barbital ranged from 100.1 to 101.4 per cent with an average of 100.7 per cent. The recoveries of aminopyrine ranged from 99.3 to 100.8 per cent with an average of 99.6 per cent. In four analyses by the second method, the barbital recovered ranged from 97.9 to 101.8 per cent with an average of 100.3 per cent and the aminopyrine recoveries from final assays ranged from 98.8 to 100.7 per cent with an average of 99.5 per cent.

Separation of Aminopyrine and Phenobarbital.—Mixtures of aminopyrine and phenobarbital with and without starch were prepared. These were assayed by each of the two methods previously described for barbital mixtures, except that two of the trials were made with *N* hydrochloric acid.

The recoveries for phenobarbital by the first method from two trials using *N* hydrochloric acid were 109.0 per cent and 106.0 per cent. The recoveries for aminopyrine in the same trials were 99.8 and 98.8 per cent. The recoveries for phenobarbital from seven assays in 5 per cent sulfuric acid medium ranged from 99.8 per cent to 103.8 per cent; average 101.6 per cent. Recoveries for aminopyrine in the same tests ranged from 99.0 to 100.0 per cent; average 99.7 per cent.

The recoveries in five assays for aminopyrine, using the second method, ranged from 99.5 to 100.2 per cent; average 99.8 per cent. Recoveries of phenobarbital in the same tests ranged from 99.2 to 107.6 per cent; average 101.5 per cent.

Separation of Aminopyrine, Antipyrine and Caffeine.—A standard mixture of aminopyrine, antipyrine, anhydrous caffeine and starch was prepared and the preparation was assayed by the method previously described for the separation of aminopyrine from caffeine. The method was applied to the separation of the aminopyrine from the total antipyrine and caffeine, but no attempt was made to separate the antipyrine and caffeine since the Association of Official Agricultural Chemists (15) has already adopted a method for this purpose.

In six experiments the recoveries for total antipyrine and caffeine ranged from 100.0 to 101.3 per cent; average 100.7 per cent. For aminopyrine the recoveries ranged from 98.9 per cent to 102.3 per cent; average 100.2 per cent.

Separation of Aminopyrine and Cinchophen.—Theoretically, the method used for the separation of aminopyrine from caffeine should be applicable to the separation of aminopyrine and cinchophen. However, owing to the relatively low solubility of cinchophen in chloroform (1 Gm. in 400 cc.), it was found expedient to use a mixture of chloroform and ether (2 + 1) instead of chloroform for its extraction. Two methods were tried as in the assay of barbital.

In four assays made according to the first method on a sample containing 40.63 per cent cinchophen and 18.87 per cent aminopyrine, the recoveries for cinchophen ranged from 99.6 to 100.5 per cent with an average of 100 per cent and those for aminopyrine ranged from 98.5 to 101.9 per cent with an average of 99.6 per cent.

Five assays made by the second procedure on the same sample gave recoveries of cinchophen ranging from 99.1 to 103.0 per cent with an average of 100.7 per cent, and those for aminopyrine ranging from 99.7 to 104.3 per cent with an average of 101.7 per cent.

Separation of Aminopyrine, Acetophenetidin, Caffeine and Phenobarbital in Mixtures.—The results from the separation of aminopyrine from caffeine, acetophenetidin, barbital, cinchophen and phenobarbital having been reasonably successful, it was decided to try the separation of four medicinal in-

Table III.—Analyses of Aminopyrine, Phenobarbital, Caffeine and Acetophenetidin Mixtures

	Aminopyrine			Phenobarbital			Caffeine			Acetophenetidin		
Sample	Found, %	Theory, %	Recovery, %	Found, %	Theory, %	Recovery, %	Found, %	Theory, %	Recovery %	Found, %	Theory, %	Recovery, %
1	47.18	47.23	99.9	2.00	2.03	98.5	4.24	4.06	104.4	23.87	24.34	98.1
2	47.27	47.23	100.1	1.95	2.03	96.1	4.12	4.06	101.5	24.05	24.34	98.8
3	46.92	47.23	99.3	1.98	2.03	97.5	4.11	4.06	101.2	24.33	24.34	100.0
4	45.15	45.08	100.2	2.13	2.13	100.0	5.51	5.19	106.0	24.14	24.78	97.4
5	45.01	45.08	99.8	2.19	2.13	102.8	5.21	5.19	100.4	25.58	24.78	103.2
6	45.20	45.08	100.3	2.09	2.13	98.1	5.13	5.19	98.8	25.19	24.78	101.7
7	45.24	45.08	100.4	2.16	2.13	101.4	5.09	5.19	98.1	25.41	24.78	102.5
8	43.74	45.08	97.0	2.06	2.13	96.7	5.18	5.19	99.8	25.47	24.78	102.8
9	45.93	45.08	101.9	2.11	2.13	99.1	5.08	5.19	97.9	23.32	24.78	94.1
10	45.29	45.08	100.5	2.23	2.13	104.7	5.21	5.19	100.4	24.71	24.78	99.7
11	44.15	45.08	97.9	2.11	2.13	99.1	5.25	5.19	101.2	24.56	24.78	99.1
Average	45.55	45.67	99.7	2.09	2.10	99.5	4.92	4.88	100.8	24.60	24.66	99.8

gredients when mixed. Accordingly, a mixture containing aminopyrine, caffeine, acetophenetidin and phenobarbital with starch[2] was prepared. This was subjected to the following method:

Aminopyrine.—About 5 Gm. of the powdered tablet mixture was weighed into a separator, 25 cc. of *N* sulfuric acid and 75 cc. of chloroform added and the mixture well shaken. The chloroform was drawn off and washed successively with 10 cc. of 3.5 per cent sulfuric acid and 10 cc. of 1 per cent hydrochloric acid. The chloroform was filtered into a weighed Erlenmeyer flask, the mixture in the first separator extracted with six more portions of 50 cc. each of chloroform, each portion washed successively through the acidified waters as before and collected in the weighed flask. This chloroform solution was retained for the recovery of acetophenetidin, caffeine and phenobarbital. The acid washings in the second and third separators were added to the first separator, the mixture made alkaline with ammonia T.S. and the aminopyrine removed by successive extractions with 25-cc. portions of chloroform. The chloroform extracts were washed in a second separator with 5 cc. of water containing a few drops of ammonia T.S. and the solvent filtered through cotton into a tared flask. Most of the solvent was recovered and the remainder evaporated while the container was rotated in an inclined position. A few cc. of anhydrous ether were added and the mixture again evaporated. The residue was dried at 80° and weighed as aminopyrine.

Phenobarbital.—The chloroformic solution of acetophenetidin, etc., was evaporated to 50 cc. and shaken 5 times with 25-cc. portions of 0.1*N* sodium hydroxide which removed phenobarbital as the soluble sodium compound. The aqueous alkaline solutions were drawn off and each washed with 5 cc. of chloroform to remove the last traces of acetophenetidin and caffeine. The chloroformic washings were added to the original chloroformic solution of caffeine and acetophenetidin. The aqueous, alkaline solution was acidified and shaken with chloroform-ether (2 + 1) to remove the phenobarbital.[3] The solvent was washed, evaporated and the residue of phenobarbital dried and weighed in the usual manner.

Acetophenetidin and Caffeine.—Acetophenetidin and caffeine were separated according to method described by Grove (16).

The results of the analysis of two prepared samples by the above technique are given in Table III.

COMMENTS

Of the various methods of extraction tried, the most satisfactory was the removal of all of the drugs except aminopyrine by shaking with chloroform or chloroform-ether mixture in the presence of 5 per cent sulfuric acid. The aminopyrine remained in the acid solution and was removed by chloroform after the addition of alkali.

Traces of aminopyrine were persistently carried over in the chloroform extractions from acid solutions. This is shown by the generally high results obtained for acetophenetidin, barbital, caffeine, phenobarbital, etc., and by the faintly yellowish color of these residues when heated for some time at 100°. The presence of aminopyrine in these fractions usually may be shown by heating the residues of caffeine, phenobarbital, etc., with water, cooling, filtering and adding a few drops of cadmium iodide solution to the filtrate. In most cases, however, the error due to the extraction of aminopyrine from acid solutions was negligible.

SUMMARY

Two quantitative methods are described in this paper but both are not applicable to all separations involving aminopyrine. Methods have been developed for the determination of aminopyrine, acetophenetidin, antipyrine, barbital, caffeine, cincho-

[2] Tablets of this approximate composition are on the market.

[3] An automatic extractor may be used to advantage for this operation.

phen and phenobarbital in mixtures and by a combination of these methods with A. O. A. C. procedures, determinations of as many as four of the constituents in the same mixture have been made. The recoveries by the recommended methods range from about 98 per cent to about 104 per cent.

Precipitation of the aminopyrine as the double salt with cadmium iodide or with mercuric chloride was not entirely satisfactory. Attempts to separate the several substances by the differences in their solubilities in various solvents were not satisfactory.

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NOTICE

Send in papers for Richmond meeting to Section Secretaries by March 15th.

The Determination of Camphor in Camphor Liniment

An Accurate and Simplified Volatilization Method

*By Solomon M. Berman**

The last three revisions of the U. S. Pharmacopœia have presented as many different assay methods for Liniment of Camphor. The U. S. P. IX method involved removal of the camphor from the oily vehicle by means of hot alcohol vapor, the camphor in the distillate being measured polariscopically. The U. S. P. X assay was essentially that proposed by Miller (1) who stated that 90 minutes heating at 110° in a platinum dish was sufficient to volatilize the camphor, without decomposing the oil. He criticized the earlier method as impractical. Previously, Cook (2) had suggested a 3-hour heating at 100° for removing 97.5% of the camphor in a 20% liniment, with a suitable correction factor. Lothian (3) recommended the use of petri dishes and heating on the water-bath for one hour. He noted that the residue gained in weight on further heating, while the oil, used in preparing the liniment, did not gain. He concluded that blanks were not dependable.

In a series of papers, Poe and others (4) made a detailed report of studies on the assay of Camphor Liniment. They found that the U. S. P. X method gave low results on the official liniment as well as on liniments prepared from the other common fixed vegetable oils. They also observed that aluminum dishes gave the lowest results in any series of determinations, while glass dishes were relatively satisfactory and tin or lead vessels were best. These workers found that the most serious error in the volatilization procedure was due to oxidation of the oil at the temperature used, the effect increasing with time and more pronounced when aluminum vessels were employed. Subsequent studies of heating *in vacuo*, in nitrogen, in carbon dioxide, and in the presence of anti-oxidants, led to their

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