A STUDY OF ISOPROPYL ETHER AND METHYLENE CHLORIDE AS SOLVENTS IN ALKALOIDAL ASSAYING, II.*,[1]

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INTRODUCTION.

During the past few years a considerable number of new solvents have been introduced by the chemical industry, many of which are commercially available in such quantities as to make them economically useful for many purposes. Many of these modern solvents have been used in certain pharmaceutical operations, such as the extraction of oils, fats, waxes, resins, balsams and other plant and animal principles.

Several excellent articles (1, 2, 3) have appeared in recent years on the general subject of new solvents and their uses. However, no study of the value of any of these solvents in extracting alkaloids quantitatively from vegetable drugs has been reported. It is the purpose of this investigation, therefore, to determine the value of certain of these solvents in the quantitative determination of the alkaloidal content of certain drugs.

For several reasons isopropyl ether and methylene chloride have been selected for this study. In the first place, these solvents are sufficiently insoluble in water to be classified under the general heading of "immiscible solvents." Also, the isopropyl ether possesses properties somewhat similar to ethyl ether, which is now used in many of the assay processes, with certain additional advantages, such as lower vapor pressure, higher boiling point, higher flash point, less solubility in water and in many cases somewhat higher solvent power. Methylene chloride has a lower boiling point than chloroform, and a specific gravity of about 1.33 as compared to 1.48 for chloroform. It is also practically insoluble in water. Thus, either one of these solvents may be used alone, or combined with the other in any proportion, as immiscible substances to extract alkaloids from aqueous solution.

Upon consideration of the many factors involved in alkaloidal assaying, few generalizations in regard to the value of a solvent can be made with any degree of accuracy. The solubility of the alkaloid or alkaloids in a given solvent is not always a criterion of its usefulness in extracting the alkaloids from vegetable drugs. The physical properties of the powdered drug may be such as to make it difficult to extract the alkaloids in a given time, while, on the other hand, the nature of the solvent may be such that it will easily penetrate the cell walls and thus prove to be highly efficient in dissolving out the alkaloids.

It is for these and other reasons that a comparative study of isopropyl ether and methylene chloride with those solvents now used in certain official assay processes has been undertaken.

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* Presented before the Scientific Section, A. PH. A., New York meeting, 1937.

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The drugs selected for study were Belladonna, Cinchona, Nux Vomica and Guarana. The assay procedures as given in the United States Pharmacopœia X have been strictly followed, with no attempt to alter the methods of procedure in any way.

EXPERIMENTAL.

Belladonna Leaves (*4*).—The assay procedure in the United States Pharmacopœia X is carried out by taking 10 Gm. of Belladonna Leaves in No. 60 powder, placing in a percolator of special design, and adding a sufficient amount of a 3:1 mixture of ether-chloroform to completely saturate the drug. The drug is allowed to macerate for a short period of time, and ammonia water added. After macerating for 1 hour the drug is packed firmly and a 3:1 mixture of ether-chloroform passed through the percolator slowly until the drug is extracted.

The ether-chloroform mixture is then extracted with dilute sulfuric acid, using successive 15-cc. portions of acid until the organic solvent is free from alkaloids.

The acid solution is made alkaline with ammonia and extracted with successive portions of chloroform until the aqueous layer is free from alkaloids.

Finally the chloroformic solution is evaporated to dryness, the residue taken up in a little ether and again evaporated to dryness. The residue is finally dissolved in standard sulfuric acid and the excess acid determined with standard sodium hydroxide.

Thus, it is observed that the initial solvent used to extract the alkaloids in the assay of Belladonna Leaves is a 3:1 ether-chloroform mixture, and the final solvent chloroform alone. The following tables (Tables I–VI) will show the variations in solvents, the number of extractions required and the percentage of alkaloids found.

TABLE I.—ASSAY OF BELLADONNA LEAVES.

(U. S. P. X Method.)

1st Solvent.	Acid Solution.	Number Ext'n's.	Final Solvent.	Number Ext'n's.	% Alk. Found.
3:1 ethyl ether-chloroform	H_2SO_4, 2%	4	Chloroform	4	0.308
"	"	4	"	4	0.318
"	"	5	"	4	0.312
"	"	4	"	4	0.310
				Av.	0.312

TABLE II.—ASSAY OF BELLADONNA LEAVES.

(U. S. P. X Method—Modified.)

1st Solvent.	Acid Solution.	Number Ext'n's.	Final Solvent.	Number Ext'n's.	% Alk. Found.
3:1 isopropyl ether-methylene cl.	H_2SO_4, 2%	4	Methylene chloride	4	0.142
"	"	4	"	4	0.154
"	"	4	"	4	0.152
"	"	4	"	4	0.147
				Av.	0.149

TABLE III.—ASSAY OF BELLADONNA LEAVES.

(U. S. P. X Method—Modified.)

1st Solvent.	Acid Solution.	Number Ext'n's.	Final Solvent.	Number Ext'n's.	% Alk. Found.
3:1 ethyl ether-chloroform	H_2SO_4, 2%	4	Methylene chloride	4	0.303
"	"	4	"	4	0.310
"	"	4	"	4	0.307
"	"	4	"	4	0.318
				Av.	0.309

TABLE IV.—ASSAY OF BELLADONNA LEAVES.

(U. S. P. X Method—Modified.)

1st Solvent.	Acid Solution.	Number Ext'n's.	Final Solvent.	Number Ext'n's.	% Alk. Found.
3:1 ethyl ether-methylene cl.	H_2SO_4, 2%	4	Methylene chloride	4	0.294
"	"	4	"	4	0.283
"	"	4	"	4	0.290
"	"	4	"	4	0.279
				Av.	0.284

TABLE V.—ASSAY OF BELLADONNA LEAVES.

(U. S. P. XI Method—Modified.)

1st Solvent.	Acid Solution.	Number Ext'n's.	Final Solvent.	Number Ext'n's.	% Alk. Found.
10 cc. alcohol 20 cc. ether	H_2SO_4, 0.1N	4	Chloroform	4	0.318
"	"	4	"	4	0.312
"	"	4	"	4	0.328
"	"	4	"	4	0.301
				Av.	0.315

TABLE VI.—ASSAY OF BELLADONNA LEAVES.

(U. S. P. XI Method—Modified.)

1st Solvent.	Acid Solution.	Number Ext'n's.	Final Solvent.	Number Ext'n's.	% Alk. Found.
10 cc. alcohol 20 cc. isopropyl ether	H_2SO_4, 0.1N	4	Chloroform	4	0.292
"	"	4	"	4	0.268
"	"	4	"	4	0.272
"	"	4	"	4	0.284
				Av.	0.279

The sample of Belladonna Leaves used for the determinations recorded in Tables I–VI, inclusive, was in a No. 60 powder and was labeled U. S. P. The average of four determinations is recorded in each case. These determinations were run in duplicate, the first pair being numbered 1, 2 and the second pair 3, 4 in the tables. In some cases more than four determinations were made and the four in closest agreement selected.

Table I will show the results obtained when assayed according to the U. S. P. X Method. It will be observed that in three cases four extractions were required with dilute sulfuric acid and in one case five extractions. The number of extractions will depend somewhat upon the operator; however, it is safe to say that four extractions with 15-cc. portions of 2 per cent sulfuric acid are sufficient for complete extraction in most cases. It will also be observed that four extractions with chloroform (25, 20, 15, 15 cc.) are sufficient for complete extraction of the alkaloids from the alkaline aqueous solution.

The results in Table II were obtained by extracting the drug with a 3:1 mixture of isopropyl ether-methylene chloride, and using methylene chloride as the final immiscible solvent. The low results obtained are, no doubt, largely due to the inability of the isopropyl ether to penetrate the powdered drug and allow the mixed solvent to completely extract the alkaloids. This conclusion is substantiated by an examination of the results in Table IV, where the initial solvent is a 3:1 mixture of ethyl ether-methylene chloride and the final solvent methylene chloride.

Table III shows the results obtained when an initial solvent of 3:1 ether-chloroform was used, and methylene chloride as the final immiscible solvent. Upon examination of these results it is clear that methylene chloride is equally as efficient as chloroform for extracting atropine from aqueous solution.

Table IV shows the results obtained when a 3:1 mixture of ethyl ether-methylene chloride and a final solvent of methylene chloride was used. It is observed that methylene chloride sub-

stituted for chloroform in the initial solvent is not quite as efficient as the latter when used in the same proportion.

Table V indicates the results obtained when the same sample of Belladonna Leaves was assayed by the continuous extraction method as outlined in the U. S. P. XI. Table VI shows the results obtained when isopropyl ether was substituted for ethyl ether in the above procedure.

From an analysis of the results in Tables I–VI, inclusive, it is seen that isopropyl ether is not as efficient a solvent as ethyl ether in the assay of Belladonna Leaves, according to the U. S. P. X or U. S. P. XI assay methods. However, methylene chloride is shown to be as efficient as chloroform for removing the alkaloids from the alkaline aqueous solution in the final extraction, and might, therefore, be used instead of chloroform for this purpose.

Cinchona (5).—Cinchona was selected because it represents an official drug assay gravimetrically by Type Process A of the U. S. P. X. The powdered drug used in the assays was in a No. 60 powder and labeled U. S. P.

The steps in the assay of Cinchona for total alkaloids are as follows: The drug is heated for one hour on a water-bath with a small amount of diluted hydrochloric acid and distilled water. A 3:1 ether-chloroform mixture is then added and followed by ammonia water to render the mixture alkaline. The alkaline liquid is then shaken intermittently during two hours or, in a mechanical shaker for one hour. After standing over night, the mixture is again shaken for one-half hour.

An aliquot portion of the liquid is decanted, representing a definite weight of the drug, and transferred to a separator. The alkaloids are extracted from the organic solvent with successive 15-cc. portions of 2 per cent sulfuric acid.

The acid solution, containing the total alkaloids in the form of sulfates, is made alkaline with ammonia water and completely extracted with chloroform. Finally the chloroform extract is evaporated to dryness, the residue dried to constant weight at 100° C. and weighed.

The data recorded in Tables VII–XIV, inclusive, were obtained by following strictly the assay procedure in the U. S. P. X, the only change being in the nature of the solvents used, as reported in the tables.

TABLE VII.—ASSAY OF CINCHONA FOR TOTAL ALKALOIDS.

(U. S. P. X Method.)

1st Solvent.	Acid Solution.	Number Ext'n's.	Final Solvent.	Number Ext'n's.	% Alk. Found.
3:1 ethylether-chloroform	H_2SO_4, 2%	5	Chloroform	7	7.96
"	"	4	"	7	7.84
"	"	4	"	7	7.92
"	"	4	"	7	7.87
				Av.	7.89

TABLE VIII.—ASSAY OF CINCHONA FOR TOTAL ALKALOIDS.

(U. S. P. X Method—Modified.)

1st Solvent.	Acid Solution.	Number Ext'n's.	Final Solvent.	Number Ext'n's.	% Alk. Found.
3:1 isopropyl ether-methylene cl.	H_2SO_4, 2%	4	Chloroform	6	3.30
"	"	4	"	6	3.24
"	"	4	"	5	3.14
"	"	4	"	6	3.20
				Av.	3.22

TABLE IX.—ASSAY OF CINCHONA FOR TOTAL ALKALOIDS.

(U. S. P. X Method—Modified.)

1st Solvent.	Acid Used.	Number Ext'n's.	Final Solvent.	Number Ext'n's.	% Alk. Found.
3:1 isopropyl ether-methylene cl.	H_2SO_4, 2%	4	Methylene chloride	6	3.37
"	"	4	"	6	3.28
"	"	4	"	6	3.19
"	"	4	"	6	3.31
				Av.	3.28

TABLE X.—ASSAY OF CINCHONA FOR TOTAL ALKALOIDS.

(U. S. P. X Method—Modified.)

1st Solvent.	Acid Used.	Number Ext'n's.	Final Solvent.	Number Ext'n's.	% Alk. Found.
3:1 isopropyl ether-chloroform	H_2SO_4, 2%	4	Methylene chloride	6	3.47
"	"	4	"	6	3.55
"	"	4	"	6	3.44
"	"	4	"	6	3.48
				Av.	3.51

TABLE XI.—ASSAY OF CINCHONA FOR TOTAL ALKALOIDS.

(U. S. P. X Method—Modified.)

1st Solvent.	Acid Used.	Number Ext'n's.	Final Solvent.	Number Ext'n's.	% Alk. Found.
3:1 ethyl ethyl-chloroform	H_2SO_4, 2%	4	Methylene chloride	6	7.81
"	"	4	"	6	7.71
"	"	4	"	6	7.79
"	"	4	"	6	7.87
				Av.	7.79

TABLE XII.—ASSAY OF CINCHONA FOR TOTAL ALKALOIDS.

(U. S. P. X Method—Modified.)

1st Solvent.	Acid Used.	Number Ext'n's.	Final Solvent.	Number Ext'n's.	% Alk. Found.
3:1 ethyl ether-methylene cl.	H_2SO_4, 2%	4	Methylene chloride	6	7.80
"	"	5	"	6	7.82
"	"	4	"	6	7.79
"	"	4	"	6	7.88
				Av.	7.82

TABLE XIII.—ASSAY OF CINCHONA FOR TOTAL ALKALOIDS.

(U. S. P. X Method—Modified.)

1st Solvent.	Acid Used.	Number Ext'n's.	Final Solvent.	Number Ext'n's.	% Alk. Found.
3:1 ethyl ether-chloroform 5% alcohol	H_2SO_4, 2%	4	Chloroform	6	8.22
"	"	4	"	6	8.31
"	"	4	"	6	8.28
"	"	4	"	6	8.14
				Av.	8.23

TABLE XIV.—ASSAY OF CINCHONA FOR TOTAL ALKALOIDS.

(U. S. P. X Method—Modified.)

1st Solvent.	Acid Used.	Number Ext'n's.	Final Solvent.	Number Ext'n's.	% Alk. Found.
3:1 ethyl ether-methylene cl. 5% alcohol	H_2SO_4, 2%	4	Methylene chloride	6	8.12
"	"	4	"	6	8.02
"	"	4	"	6	8.09
"	"	4	"	6	8.04
				Av.	8.06

Table VII shows the results obtained when the Cinchona was assayed according to the U. S. P. Method. It required four extractions in three cases and five extractions in one case, using 15-cc. portions of sulfuric acid. Seven extractions were required with chloroform in every case to completely remove the alkaloids from the alkaline aqueous solution, using 25, 20, 15 . . . 15 cc.

Table VIII is a summary of the results obtained when a 3:1 mixture of isopropyl ether-methylene chloride is used to extract the drug and chloroform to remove the alkaloids from the alkaline aqueous solution. From the amount of alkaloids obtained it is clear that such a mixture of isopropyl ether-methylene chloride is not suited as a solvent to extract cinchona alkaloids from the crude drug. The low results obtained are due to the inefficiency of the isopropyl ether and not to the methylene chloride, as may be seen from an examination of Table XII. The same number of extractions with dilute sulfuric acid are required to remove the alkaloids from the organic solvent as were used in the experiments recorded in Table VII. From a consideration of the distribution ratio of quinine between these two liquids this is to be expected.

Table IX indicates the results obtained when the initial solvent was a 3:1 mixture of isopropyl ether-methylene chloride, and the final solvent methylene chloride. The only conclusion that can be drawn from the data is that methylene chloride seems to be about as efficient as chloroform for removing the cinchona alkaloids from alkaline aqueous solution.

Table X shows the results obtained when a 3:1 mixture of isopropyl ether-methylene chloride was used to extract the alkaloids from the drug and methylene chloride to remove the alkaloids from alkaline aqueous solution. As in Table IX, it may be seen that isopropyl ether is not suitable for removing cinchona alkaloids from the drug quantitatively, whether it be mixed with methylene chloride or chloroform.

The experiments carried out and recorded in Table XI show that methylene chloride is perhaps a little more efficient than chloroform for removing the cinchona alkaloids from alkaline aqueous solution. The number of extractions necessary for the complete removal of the alkaloids by methylene chloride is six, as compared to seven when chloroform is used. However, an average of 0.1 per cent less alkaloids recovered indicates that extraction is not quite as complete with methylene chloride, even though the seventh extraction gave no test for alkaloids. Such a discrepancy in results is likely due to experimental error, because the same amounts of solvent were used in each case, namely, 25, 20, 15 . . . 15 cc., and from a consideration of the distribution ratio of quinine between water and methylene chloride, six extractions should be sufficient for the complete removal of the alkaloids.

The results in Table XII show that methylene chloride may be substituted for chloroform in the initial solvent without changing the results of the assay to any great extent. Thus, when a 3:1 mixture of ethyl ether-methylene chloride is used to extract the alkaloids from Cinchona, the amount of total alkaloids is found to be 7.82 per cent as compared to 7.79 per cent when an ethyl ether-chloroform mixture is used.

Experiments were run to show the effect of a small amount of alcohol when used with the ether-chloroform mixture and with the ether-methylene chloride mixture to extract the alkaloids from the drug. Tables XIII and XIV show that 5 per cent of alcohol mixed with either of the above solvents will extract more total alkaloids than when the solvents are used alone. When added to the ether-chloroform mixture the amount of total alkaloids obtained was 8.23 per cent, and when mixed with the ether-methylene chloride mixture 8.06 per cent was obtained.

Nux Vomica (*6*).—This drug is assayed by Type Process A of the U. S. P. X, and the alkaloids are determined volumetrically. The steps in the assay are as follows: The drug is extracted with a 3:1 ether-chloroform mixture, which has been made alkaline with ammonia, T.S., according to the general procedure under Type Process A. An aliquot portion of the liquid is collected and extracted with dilute sulfuric acid to remove the alkaloids as sulfates from the organic solvent.

The acid aqueous solution is then made alkaline with ammonia T.S., and the alkaloids completely extracted with successive portions of chloroform. Finally, the chloroform is evaporated to dryness, the residue dried at 100° C. to remove traces of ammonia and the alkaloids determined volumetrically, using methyl red, or cochineal as the indicator.

The assay procedure in the U. S. P. X was followed strictly, using a No. 60 powder in all cases. The data recorded in Tables XV–XIX, inclusive, were obtained by following this procedure, the only changes being in the solvents used.

Table XV shows the results obtained by the U. S. P. X assay method. Five extractions

TABLE XV.—ASSAY OF NUX VOMICA FOR TOTAL ALKALOIDS.

(U. S. P. X Method.)

1st Solvent.	Acid Used.	Number Ext'n's.	Final Solvent.	Number Ext'n's.	% Alk. Found.
3:1 ethyl ether-chloroform	H_2SO_4, 2%	5	Chloroform	6	2.40
"	"	5	"	6	2.48
"	"	5	"	6	2.52
"	"	5	"	6	2.45
				Av.	2.46

TABLE XVI.—ASSAY OF NUX VOMICA FOR TOTAL ALKALOIDS.

(U. S. P. X Method—Modified.)

1st Solvent.	Acid Used.	Number Ext'n's.	Final Solvent.	Number Ext'n's.	% Alk. Found.
3:1 isopropyl ether-methylene cl.	H_2SO_4, 2%	5	Chloroform	5	1.02
"	"	5	"	5	1.04
"	"	5	"	5	1.10
"	"	5	"	5	1.07
				Av.	1.05

TABLE XVII.—ASSAY OF NUX VOMICA FOR TOTAL ALKALOIDS.

(U. S. P. X Method—Modified.)

1st Solvent.	Acid Used.	Number Ext'n's.	Final Solvent.	Number Ext'n's.	% Alk. Found.
3:1 ethyl ether-methylene cl.	H_2SO_4, 2%	5	Chloroform	5	1.11
"	"	5	"	5	1.07
"	"	5	"	6	1.16
"	"	5	"	5	1.08
				Av.	1.10

TABLE XVIII.—ASSAY OF NUX VOMICA FOR TOTAL ALKALOIDS.

(U. S. P. X Method—Modified.)

1st Solvent.	Acid Used.	Number Ext'n's.	Final Solvent.	Number Ext'n's.	% Alk. Found.
3:1 isopropyl ether-methylene cl.	H_2SO_4, 2%	4	Methylene chloride	6	0.102
"	"	4	"	6	0.092
"	"	4	"	6	0.090
"	"	4	"	6	0.084
				Av.	0.092

TABLE XIX.—ASSAY OF NUX VOMICA FOR TOTAL ALKALOIDS.

(U. S. P. X Method—Modified.)

1st Solvent.	Acid Used.	Number Ext'n's.	Final Solvent.	Number Ext'n's.	% Alk. Found.
45% isopropyl ether 50% chloroform 5% alcohol	H_2SO_4, 2%	4	Chloroform	6	1.45
"	"	4	"	6	1.52
"	"	4	"	6	1.58
"	"	4	"	6	1.48
				Av.	1.51

with 15-cc. portions of 2 per cent sulfuric acid were required to completely remove the alkaloids from the mixed organic solvent, and six extractions to remove them from the alkaline aqueous solution. The sample of Nux Vomica determined showed an average alkaloid content of 2.46 per cent.

Table XVI indicates the efficiency of isopropyl ether-methylene chloride mixture when used to extract the alkaloids from Nux Vomica. The amount of alkaloids found is less than 50 per cent of the amount shown to be present by the U. S. P. method. It is clear, therefore, that a 3:1 mixture of isopropyl ether-methylene chloride is not suitable as the initial solvent in extracting the alkaloids from Nux Vomica.

Ethyl ether and methylene chloride in the ratio of 3:1 parts by volume is also shown not to be a good solvent for the alkaloids of Nux Vomica. (Table XVII.) From the results obtained, it may be seen that chloroform is to be preferred over methylene chloride for this purpose. From a consideration of solubility data a mixture of three parts of isopropyl ether and one part of chloroform should extract as much of the alkaloids of Nux Vomica as a similar mixture of ethyl ether-chloroform. However, I was unable to extract over seventy per cent of the alkaloids present with this solvent. The trouble, of course, was the inability of the isopropyl ether to penetrate and soften the powdered drug to the same extent as ethyl ether, and thereby enable the chloroform to dissolve out the alkaloids.

Table XVIII shows that methylene chloride is about as efficient as chloroform for extracting Nux Vomica alkaloids from aqueous solution. Here the initial solvent is isopropyl ether-methylene chloride and the final organic solvent methylene chloride. The per cent of alkaloids found is 0.092 as compared to 1.05 in Table XVI. The addition of 5 per cent alcohol to a mixture of three volumes of isopropyl ether and one volume of chloroform increases the extractive power of the mixture somewhat, however, the amount of alkaloids extracted amounts to only about sixty per cent of that obtained when a mixture of ethyl ether-chloroform is used. (Table XIX.)

From a consideration of the data in Tables XV–XIX, inclusive, it is clearly shown that isopropyl ether is not as efficient as ethyl ether when used with chloroform or methylene chloride to extract the alkaloids from Nux Vomica. It is also evident that methylene chloride may be used in place of chloroform in the assay, whether to extract the alkaloids from the crude drug, or to remove them from aqueous solution in a later step of the assay. Methylene chloride possesses the apparent additional advantage over chloroform of forming less troublesome emulsions when shaken with aqueous alkaline solutions. In many of the assays run it was noticeable that methylene chloride forms less permanent emulsions under approximately the same conditions. This observation, however, needs to be investigated further.

TABLE XX.—ASSAY OF GUARANA FOR CAFFEINE.

(N. F. V Method.)

1st Solvent	Acid Used.	Amount Acid Used.	Final Solvent.	Number Ext'n's.	% Alk. Found.
Chloroform	H_2SO_4, 1%	10 cc.	Chloroform	6	4.12
"	"	10 cc.	"	6	4.19
"	"	10 cc.	"	6	4.14
"	"	10 cc.	"	6	4.21
				Av.	4.16

TABLE XXI.—ASSAY OF GUARANA FOR CAFFEINE.

(N. F. V Method—Modified.)

1st Solvent.	Acid Used.	Amount Acid Used.	Final Solvent.	Number Ext'n's.	% Alk. Found.
Methylene chloride	H_2SO_4, 1%	10 cc.	Methylene chloride	6	4.15
"	"	10 cc.	"	6	4.08
"	"	10 cc.	"	6	4.27
"	"	10 cc.	"	6	4.34
				Av.	4.21

Guarana (7).—The sample of Guarana used for analysis was labeled N. F. V and was in a No. 60 powder. When assayed by the N. F. V procedure, 4.16 per cent of caffeine was found to be present. (Table XX.) Since chloroform is the solvent used to extract the alkaloid from the drug according to the official procedure, and also, since caffeine is not soluble in isopropyl ether to any great extent, it was decided to substitute methylene chloride alone for chloroform in the assay. Table XXI shows the results obtained when methylene chloride is used in place of chloroform as the initial solvent, and also as the immiscible solvent to extract the caffeine from the alkaline aqueous solution. An average of 4.21 per cent caffeine shows that methylene chloride may be substituted for chloroform in this assay. Six extractions with methylene chloride were required to completely remove the caffeine from the alkaline aqueous solution. This is the same as when chloroform is used; therefore from the standpoint of time required methylene chloride is equally efficient, but no more so, than chloroform for this purpose.

SUMMARY AND CONCLUSIONS.

1. It has been shown that a 3:1 mixture of isopropyl ether-methylene chloride is not as efficient as a 3:1 mixture of ethyl ether-chloroform for extracting the alkaloids from the drug in the assay of Belladonna Leaves. It has also been shown that methylene chloride is not quite as good as chloroform for the same purpose; however, it has been established that methylene chloride is about as efficient as chloroform for removing the alkaloids from aqueous solution in the final extraction with immiscible solvent. (Tables I–VI.)

2. In the assay of Cinchona, isopropyl ether cannot be substituted for ethyl ether to extract the alkaloids from the drug; however, methylene chloride may be used in place of the chloroform. It has been found that methylene chloride is equally as efficient as chloroform, for removing the alkaloids from the aqueous layer, in the final extraction of cinchona alkaloids. Alcohol increases the amount of material extracted when used with ethyl ether-chloroform or ethyl ether-methylene chloride in extracting the drug. (Tables VII–XIV.)

3. Isopropyl ether is not as efficient as ethyl ether when used with chloroform or methylene chloride to extract the alkaloids from Nux Vomica. Methylene chloride may be used as a substitute for chloroform in the assay, either to extract the alkaloids from the crude drug or to remove them from the aqueous layer in the final extraction step of the assay. Less troublesome emulsions were encountered when methylene chloride was used to extract nux vomica alkaloids from alkaline aqueous solution than when chloroform was used. (Tables XV–XIX.)

4. Methylene may be used as a substitute for chloroform in the assay of Guarana. (Tables XX–XXI.)

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(5) *Ibid.*, page 109.
(6) *Ibid.*, page 246.
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