

THE ADSORPTION ANALYSIS OF TROPA ALKALOIDS

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THE sorption of alkaloids has been the subject of several papers. Alumina has been employed for the purification of extracts, etc. Ion exchange resins have been used in order to remove alkaloids from a solution, but it has been difficult to elute them quantitatively from the resin.¹⁻¹¹ Permutits and even carbon have also been tried.^{12,13,14,15}

In the course of an investigation of the analysis of preparations containing different tropa alkaloids it was necessary to purify the final alkaloidal solution prior to the estimation. For this purpose decalso* was chosen. Decalso is a synthetic silicate of the permutit type and can be obtained in a fairly uniform quality. It has been used in vitamin assays,^{16,17} e.g., for the purification of extracts containing aneurine which is taken up quantitatively by the decalso and easily removed.

In the present paper it is shown that the same applies to tropa alkaloids. The alkaloids are completely removed from their solutions and can easily be eluted in quantitative yields. The tertiary and quaternary alkaloids behave similarly, but tropic acid, a decomposition product of them, is not adsorbed under the experimental conditions used. Hence, it is possible to estimate the alkaloids even in partly decomposed preparations. In combination with the method of assay used for the final determination the procedure arrived at is specific, accurate, and rapid.

PROCEDURE RECOMMENDED

Reagents.

Decalso F was activated by alternate washing with potassium chloride and acetic acid according to *Vitamin Assay*.¹⁶ The dried material was sifted on a 200 mesh sieve and the dust discarded. In some batches of the crude material it was necessary to repeat the washing several times, as the decalso contained a small amount of iron which was difficult to remove. This contaminant is eluted by hydrochloric acid in the standard procedure and interferes with the spectrophotometric determination of the alkaloids. During the preliminary experiments it was found that decalso could be used 2 or 3 times, but after that the recoveries decreased rapidly.¹⁸ Therefore fresh decalso was used in each experiment.

Sodium hydroxide, Hydrochloric acid, Acetic acid.

Solutions of different strengths. The solutions should be made from reagent grade chemicals and stored in resistant glass containers to avoid contamination with silica, iron, etc.

* Obtained from the Permutit Company, Gunnersbury Av., London, W.4.

Apparatus.

A Beckman spectrophotometer Model DU or a Hilger Model Uvispek was used.

Standard Methods.

A. Macro Scale. Prepare a suspension of about 2.5 g. of activated decalco in water (3 g. when assaying compounded preparations). To remove air it is advantageous to warm the suspension. Transfer the slurry into a tube of 7 to 8 mm. width supplied with a stopcock and a tightly packed plug of glass wool. Run off the surplus water and wash once. Pour a solution of about 15 mg. of the tropa alkaloidal salt in water on the column. The solution must not contain appreciable amounts of ionic contaminants. Let the solution flow at a rate of 1 to 2 ml./minute out of the decalco. Wash with water. Elute with 0.2 N hydrochloric acid, collecting 20 ml. quantities of eluate in two 25-ml. flasks. Usually all the alkaloid is found in the first eluate. Add 1.5 ml. of 5N sodium hydroxide to each flask and heat for about 10 to 15 minutes on a water bath. Add 2.0 ml. of 5N hydrochloric acid which will dissolve the flocculent precipitate, cool, make up to 25 ml. and measure the extinction at 254.0, 257.5 and 261.5 $m\mu$, as described by Berggren and Nordberg.¹⁹ When working on an ethereal solution of the alkaloidal base the decalco column should be washed with ether prior to the adsorption. Instead of the hydrochloric acid 0.2 N acetic acid can be used. Here, however, some alkaloid will be present in the second eluate.

B. Micro Scale. Prepare a suspension of 0.25 g. of decalco as above (0.3 g when assaying compounded preparations). The tube should have a width of 4 to 5 mm. and the "dead space" under the stopcock should be as small as possible. Introduce about 1.5 mg. of the alkaloid as above. Elute with 0.2N hydrochloric acid at a rate not exceeding 0.5 ml./minute, collecting about 4 ml. in a 5-ml. flask. Add 0.3 ml. of 5N sodium hydroxide, hydrolyse as above, add 0.4 ml. of 5M hydrochloric acid and make up to 5 ml. Measure the extinctions in 2 cm. cells.

EXPERIMENTAL

Preliminary experiments showed that 2 g. of decalco was necessary to secure complete adsorption of 15 mg. of alkaloid by a column 7 to 10 mm. wide. More decalco requires more eluant for the complete elution and the eluate will be unnecessarily diluted. However, as the activation of the adsorbent might turn out slightly differently and it might be difficult to get a constant flow through the column, we preferred to use 2.5 g. of decalco—or even 3 g., when assaying compounded preparations.

For the experiments the following alkaloids were used, all dried *in vacuo*: atropine sulphate, conforming to the Swedish pharmacopœia Ed. XI. 97.5 per cent. according to the spectrophotometric assay¹⁹; hyoscine hydrobromide, Ph. Sv. Ed. XI, 99.5 per cent.; atropine methobromide, Ph. Sv. Ed. XI, 99.5 per cent., and hyoscine methonitrate,

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made by Pharmacia Ltd., 99.8 per cent. Solutions containing about 15 mg. of the alkloid per 5 or 10 ml. were prepared. 5 or 10 ml. was treated by the proposed method and simultaneously assayed directly by the spectrophotometric method. In this way, all weighing and many measuring errors were eliminated. It was established that the four

TABLE I
RECOVERIES ON ELUTION FROM DECALSO, MACRO SCALE

Alkaloid	Decalco g.	Eluate	Recovery, per cent.			
			First 20 ml. of eluate	Second 20 ml. of eluate	Third 20 ml. of eluate	Total
Atropine sulphate	2.5	0.2 N hydrochloric acid	97.9	1.0	0	98.9
	2.5	"	98.3	1.0	0	99.3
	3.0	"	100.2	0	0	100.2
	2.5	0.2 N acetic acid	98.0	1.1	0.9	100.0
	2.5	"	99.4	1.4	0.5	101.3
	3.0	0.1 "	85.3	16.8	0	102.1
	3.0	"	86.9	13.5	0	100.4
	3.0	0.01 "	6.8	58.2	37.6*	102.6
	3.0	5 per cent. potassium chloride	74.5	18.7	0	93.2
	3.0	"	77.6	16.1	0	93.7
	3.0	25 "	88.2	9.4	0	97.6
	3.0	" in 0.1 N acetic acid	86.5	11.2	0	97.7
3.0	"	78.3	16.5	0	94.8	
Atropine base in ether	3.0	0.2 N hydrochloric acid	98.8	0	0	98.8
Hyoscine hydrobromide	3.0	"	93.5	6.0	0	99.5
	2.5	"	100.2	0	0	100.2
	2.5	"	100.2	0	0	100.2
	2.5	"	99.7	0	0	99.7
	2.5	"	100.7	0	0	100.7
	2.5	"	98.9	0	0	98.9
	2.5	0.2 N acetic acid	97.0	1.7	0	98.7
Hyoscine base in ether	2.5	0.2 N hydrochloric acid	96.3	1.8	0	98.1
	2.5	"	97.6	0	0	97.6
	2.5	"	96.5	0	0	96.5
	2.5	"	99.0	0	0	99.0
Atropine methobromide	2.0	"	99.2	0	0	99.2
	2.5	"	99.1	2.4	0	101.5
	2.5	"	99.4	2.6	0	102.0
	2.5	0.2 N acetic acid	85.0	11.5	1.5	98.0
	2.5	"	83.8	12.2	0	96.0
3.0	25 per cent. potassium chloride	97.6	0	0	97.6	
Hyoscine methonitrate	3.0	"	92.0	4.9	0	96.9
	2.5	0.2 N hydrochloric acid	98.8	1.1	0	99.9
	2.5	"	99.9	0	0	99.9
	2.5	"	97.2	2.3	0	99.5
	2.5	"	96.1	2.5	0	98.6
	2.5	"	95.8	5.0	0	100.8
	4.0	"	82.0	19.0	0	101.0
	3.0	25 per cent. potassium chloride	95.7	0	0	95.7

* Elution with 0.1N acetic acid

alkaloids were retained by the adsorbent and that they were not eluted by distilled water in quantities up to 500 ml. Likewise, they were not eluted by methanol, ethanol, acetone, or ethyl ether in great amounts. This may be seen from Table I where the recoveries after elution with the appropriate eluant is always quantitative. In many of these experiments the columns have been washed with water, in others with the organic solvents mentioned, with no resultant losses.

With the atropine and hyoscine salts the following experiments were made. Solutions containing about 15 mg. of the alkaloid were mixed with an excess of ammonia or sodium carbonate solution, and the liquids were extracted with ether, 3×30 ml., in a cylinder. The ethereal phases were siphoned off through a filter, moistened with ether, down into the column from which the water had previously been replaced by ether. After rinsing with ether, the ether was replaced by water and the alkaloids eluted. Also in these experiments the yields were total, indicating that the free alkaloidal bases were adsorbed on the decalso from ether (see Table I). That tropic acid is not adsorbed by decalso was proved by washing a solution of about 8 mg. in 20 ml. of water through 4 g. of decalso. The recovery on elution with 40 ml. of water was 100.9 per cent. The elution of the adsorbed alkaloids was effected with hydrochloric acid, acetic acid, or potassium chloride solution.

The rate of flow through the column was usually about 1 to 2 ml./minute during the adsorption and the elution. It was found that when a high rate of flow was used during the elution the quantity of eluate needed was increased. Generally 20 ml. fractions were collected and if the rate of flow was kept sufficiently low, all the alkaloid was recovered in the first fraction when working with 0.2N hydrochloric acid. An experiment with 4 ml. fractions showed that about 75 per cent. of the alkaloid (atropine) was recovered in the first 3 fractions. When acetic acid was used, sometimes a small percentage of the alkaloids was found in the third 20 ml. fraction. The best eluant tried was 0.2N hydrochloric acid. 0.1N is also effective, but more eluate has to be taken up for a quantitative yield. Acetic acid is effective in both 0.1N and 0.2N strength, the action being inferior to that of hydrochloric acid of corresponding strength. Even with 0.01N a quantitative yield can be obtained but will, of course, give a big eluate. When working on extracts of vegetable drugs, acetic acid is superior to hydrochloric acid, as only traces of colour are eluted from the column and the background absorption in the eluate at the ultra-violet determination is greatly diminished. It is interesting to note that atropine sulphate is more easily desorbed than atropine methobromide by acetic acid. With hydrochloric acid no such difference is observed.

A 25 per cent. potassium chloride solution can also be used as eluant. Strangely enough, atropine is more difficult to desorb than methylatropine and methylhyoscine. 5 per cent. potassium chloride is far less effective as an eluant. Addition of acetic acid is without effect. As potassium chloride gives a slightly higher background absorption than acetic acid in the final ultra-violet determination when working on extracts of vegetable drugs, it has not been considered further.

0.2N hydrochloric acid was selected as the eluant in most experiments, except for extracts of vegetable drugs, when acetic acid was used. As potassium chloride eluted the alkaloids, and analyses of preparations often introduce salts into the system, the salt effect on the adsorption was investigated in the following manner. 0.008 milliequivalent of the alkaloidal salt was shaken for about 2 minutes with 3 g. of decalso in 20 ml.

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of solutions of different strengths of potassium or sodium chloride. The suspension was filtered and the alkaloid determined in suitable aliquots. The figures in Table II represent the percentages of alkaloids not adsorbed.

In order to investigate the salt effect when working in columns, atropine sulphate solution was mixed with sodium chloride solution. The sodium

TABLE II
SALT EFFECT, EQUILIBRIUM

Salt concentration Molarity	Percentages of alkaloids not adsorbed						
	Atropine sulphate		Hyoscine hydrobromide	Atropine methobromide	Hyoscine methonitrate		
	Potassium chloride	Sodium chloride	Potassium chloride	Potassium chloride	Potassium chloride		
1.0	93.8	92.4	86.6	95.4	93.7	94.6	
0.15	88.9						
0.10	88.3	85.8	82.1	86.3	85.2	81.6	
0.075	83.5						
0.050	77.6	77.8					
0.025	65.7						
0.010	50.1	51.2	53.0	47.6	48.0	45.0	44.3
0.005	32.4			52.6			
Water	23.0	24.3	29.2	22.8	19.8	18.6	

chloride concentration and the amount of adsorbent were varied, but the absolute quantity of sodium chloride was kept constant, 0.5 milliequivalent, i.e., 10 times the amount of atropine, 0.044 milliequivalent. From Table III it may be concluded that with the same amount of salt the concentration is unimportant and will cause incomplete adsorption, but if the quantity of the adsorbent is increased sufficiently the alkaloid is completely adsorbed. Hence, the presence of too much salts must be avoided.

TABLE III
SALT EFFECT, COLUMN

15 mg. of atropine sulphate + 29 mg. of sodium chloride diluted to suitable concentration (in col. 2).

Decalco g.	Salt concentration molarity	Per cent recovery after elution with			Total
		40 ml of water	20 ml 0.1 N hydrochloric acid*	20 ml 0.1 N hydrochloric acid*	
2	0.1	65.7	37.6	0	103.3
2	0.02	61.6	40.0	0	101.6
4	0.02	19.7	63.5	15.3	100.5
5	0.02	0	60.8	35.2	96.0
5	0.02	0	58.1	37.4	95.5

*Early experiments, hence the 0.1 N hydrochloric acid instead of 0.2 N.

When assaying compounded preparations it is often necessary to perform an acid extraction. In order to avoid the untoward salt effect on the adsorption we used sulphuric acid for the extraction and removed it by precipitation with barium hydroxide. To the aqueous alkaloidal extract, which is faintly acid with sulphuric acid, a few drops of methyl red is added and the solution is carefully neutralised with saturated barium hydroxide solution until a yellow colour is obtained. The red colour is restored by the addition of one drop of 0.1N sulphuric acid. The

precipitated barium sulphate is centrifuged down and the supernatant liquid decanted through the column. The precipitate is washed once or twice by centrifugation and the washings added to the column. With this method recoveries of 101.1 and 101.0 per cent. were found when working with 20 mg. of atropine sulphate and 10 milliequivalent of sulphuric acid.

When working on compounded preparations it was convenient to decrease the amount of sample as much as possible to simplify extractions, etc. For this purpose the micro standard method (B) was developed. With low flow rates the alkaloids were found in the first fraction. The recoveries are shown in Table IV.

TABLE IV
RECOVERIES ON ELUTION FROM DECALSO, MICRO SCALE

	Alkaloid	
	Taken* mg.	Found mg.
Atropine sulphate	1.48; 1.50	1.46; 1.47
Hyosine methonitrate	1.56; 1.57	1.50; 1.54; 1.51

*These figures were obtained by assaying the test solution according to Berggren and Nordberg.¹⁹

If iron had gone into the eluate it could be disposed of in the following way. After the alkaline hydrolysis on the water bath a brown precipitate of ferric hydroxide appeared. After cooling the liquid was made up to volume, the precipitate was centrifuged down, and an aliquot withdrawn, acidified, and measured.

APPLICATIONS

Solutions. Plain solutions of the tropa alkaloids can usually be assayed without adsorption. Solutions containing ethanol must be freed from it prior to the spectrophotometric determination, as pointed out by Berggren-Nordberg.¹⁹ This can, of course, be done by evaporation, but often the blanks will be high and the estimation uncertain. Moreover, there is risk of decomposition of the alkaloid. Therefore it is recommended that the standard adsorption procedure should be adopted. In this way also a possible decomposition of the sample is revealed.

Suppositories. The suppositories to be investigated contained, apart from the alkaloid, either phenobarbitone or amylobarbitone. As these substances absorb strongly in the ultra-violet region it was essential to remove them. It was found that the alkaloids could be quantitatively adsorbed from a saturated aqueous solution of these compounds, while the barbiturates were washed away, the best method being with ether. After several trials we found that the suppositories must be assayed by the micro method. The reason for this is that the alkaloids cannot be extracted from the large amount of suppository base, which is necessary for the macro method, without using inconveniently large quantities of the water phase for the extraction.

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The following mixtures were prepared:—

A. Amylobarbitone	2 g.	B. Phenobarbitone	2 g.
Hard Paraffin	8 g.	Hard Paraffin	3 g.
Oil of Theobroma	28 g.	Oil of Theobroma	37 g.
Hyoescine methonitrate	0.02103 g.	Atropine sulphate	0.02348 g.

and assayed thus.

Method 1.

3 g., accurately weighed, was melted with 25 ml. of water and shaken vigorously. When the layers had separated completely and no droplets of oil could be seen, the mixture was cooled and the water phase filtered on to 0.3 g. of decalco in the micro column. The extraction was repeated 3 times. The column was washed with a little water and then with ether until a sample of the eluate gave no visible residue after evaporation (about 20 to 30 ml.). The ether in the column was replaced with water and the analysis continued by the micro method.

Method 2.

3 g., accurately weighed, was introduced into a separating funnel together with 15 ml. of water and 30 ml. of ether. The funnel was shaken until the solid phase had dissolved. The aqueous layer was filtered down on 0.3 g. of decalco in the micro column and the extraction with water repeated 3 times. Thereafter the procedure was completed as under method 1. The results are shown in Table V.

TABLE V
ASSAY OF SUPPOSITORIES

	Alkaloid		
	Taken mg.	Found	
		Method 1 mg.	Method 2 mg.
Type A	1.64	1.54	
	1.69	1.64	
	1.99		2.09
	1.94		1.84
	1.85		1.76
Type B	1.58	1.54	
	1.80	1.92	
	1.82	1.70	
	1.75	1.66	
	1.74		1.63
	1.74		1.75

By these methods commercial suppositories with compositions similar to those given above were successfully assayed.

Tablets. Tablets containing tropa alkaloids can be assayed in two ways. In the *macro scale* the tablet powder was mixed with about 10 times its weight of water in a centrifuge tube, which was heated in boiling water to gelatinise the starch. The mixture was cooled to about 40° C. and the starch paste hydrolysed for half an hour with some saliva. After centrifugation the supernatant liquid was poured through the macro column. The residue in the tube was stirred with water, the

mixture centrifuged and the liquid again poured on the column. This was repeated once more. The column was thoroughly washed with water. If the tablets contained ether-soluble substances, e.g. barbiturates, which would interfere in the final estimation, the column was well washed with ether until a sample of the eluate did not leave a residue on evaporation. The ether in the column was replaced by water. The assay was then continued as under the standard method. However, magnesium stearate, present in some tablets, interfered strongly, presumably due to adsorption of the alkaloid on the stearate. The losses amounted to 15 to 25 per cent. It was not possible to overcome this complication.

The *micro method* solved this problem. The tablet powder was well mixed with about 30 times its weight of water in a beaker and the solution filtered into the column. The residue was repeatedly washed with small quantities of water and the procedure continued as in the macro method mentioned above. It is essential not to heat the water of extraction as the magnesium stearate otherwise seems to be wetted and will adsorb some alkaloid which cannot be recovered. Typical results are shown in Table VI.

TABLE VI
ASSAY OF TABLETS

Alkaloid	Admixture	Alkaloid	
		Taken mg.	Found mg.
Macro Assays			
Atropine sulphate . . .	1.5 g. phenobarbitone	14.58	14.40
Atropine methobromide . . .	3 g. indiff. A	15.05	14.75
Hyoscine methonitrate . . .	3 g. indiff. A	14.60	14.57
Atropine sulphate . . .	5 g. indiff. A + 1.5 g. phenobarbitone	20.00	19.97
Micro Assays			
Atropine sulphate . . .	0.5 g. indiff. B	1.39	1.33; 1.32
Hyoscine methonitrate . . .	0.5 g. indiff. B + 0.15 g. amylobarbitone	1.52	1.48; 1.42

Indiff. A = lactose + starch + talc.

Indiff. B = indiff. A + 1 per cent. of magnesium stearate.

By these methods commercial tablets, some of which contained phenobarbitone or amylobarbitone, were successfully assayed.

Extracts. In order to investigate the possibility of the proposed method for the assay of extracts the following experiments were performed (c.f. Schill and Ågren²⁰).

10.0 g. of a belladonna extract was shaken with 25 ml. of boiling water and 10 drops of 2M hydrochloric acid. After cooling, the mixture was shaken for 10 minutes with 200 ml. of ether and 10 ml. of 5M ammonia. 2.5 g. of powdered tragacanth was added and the mixture shaken again. The ether phase thus separated was filtered into a 250-ml. flask and the content made up to volume with ether washings. Of this solution aliquots were taken for assay according to a generally accepted procedure and the proposed method. The method of extraction will, of course, not give the absolute value of the alkaloidal content, but a good comparison between the two methods will be obtained.

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75.0 ml. of the ether extract was transferred to a separating funnel containing 15 ml. of 0.2N hydrochloric acid. The mixture was shaken and the aqueous phase filtered into a second separating funnel containing 20 ml. of chloroform. The acid extraction was repeated twice. The combined water phases were alkalised with ammonia and extracted with 20 + 15 + 10 ml. of chloroform, the chloroform filtered through a layer of anhydrous sodium sulphate, and evaporated to dryness. The residue was treated with a few ml. of ether and the ether evaporated. This was repeated twice. The residue was dissolved in 2 ml. of ethanol on the water bath and the solution diluted with 20 ml. of water. After cooling it was titrated with 0.01N sulphuric acid with methyl red-methylene blue as indicator. 20.00 ml. of the ether extract was assayed by the proposed macro method. After the adsorption, the column was washed with ether until colourless washings were obtained which gave no detectable residue on evaporation. The elution was effected with 0.2N acetic acid. The eluates were slightly yellowish, but this colour vanished after the final acidification and did not interfere with the estimation. The results are shown in Table VII.

TABLE VII
ASSAY OF BELLADONNA EXTRACTS

Extract	Hyoscyamine				
	Acidimetric method	Proposed method			Total
		First 20 ml. of eluate	Second 20 ml. of eluate	Third 20 ml. of eluate	
	per cent.	per cent.	per cent.	per cent.	per cent.
Ed. X Ph. Sv. with about 50 per cent. of liquorice root	0.50	0.441	0.049	—	0.50
	0.51	0.472	0.026	—	0.50
		0.456	0.039	—	0.50
Ed. XI Ph. Sv. with sodium sulphate	0.92	0.965	0.042	—	1.01
	1.05	0.933	0.040	—	0.97
		0.874	0.090	—	0.96

SUMMARY

1. Tropa alkaloids are quantitatively taken up by decalco from water and some organic solvents. They are fully desorbed by moderately strong solutions of acids or salts.
2. This applies both to tertiary and quaternary alkaloids and offers a means of isolating them.
3. In column assays either 15 or 1.5 mg. of alkaloidal salt have been assayed. The accuracy seems to be that of the final estimation used.
4. Some applications to compounded pharmaceutical preparations are given, including extracts of vegetable drugs, etc.

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