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RAPID ANALYSIS OF DOSAGE FORMS OF SOME PHARMACEUTICALS BY NONAQUEOUS THERMOMETRIC TITRIMETRY

Part 1: Analysis of some antimalarial formulations

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The use of the technique of solution thermochemistry is proposed for the rapid assay for quality control and quality assurance of dosage amounts of some ethical formulations of some antimalarial drugs. The active ingredients are chloroquine, hydroxychloroquine, dapsone, proguanil hydrochloride, and pyrimethamine.

Assay is done without the separation of the excipients and without isolation or derivatisation of the analytes. The titrations are done in glacial acetic acid and utilise the catalysed hydrolysis of acetic anhydride by perchloric acid to indicate the endpoint of the reactions.

The time taken for a typical assay, of a typical dosage amount is about 3–5 minutes. The reproducibility is of the order of 1% for the milligramme amounts of analyte present in the dosage amounts of the drugs.

The use of pharmacopoeial methods as procedures for quality control and quality assurance of dosage forms of some ethical pharmaceuticals has long been recognised as being expensive from an economic standpoint. The present cost of labour, capital equipment and general overheads make it desirable to use methods which are more rapid and less costly. Many techniques employed in the pharmacopoeial methods have to use very small amounts of the products and require careful, often tedious, separation processes in order to isolate the analyte from the excipients used for commercial purposes. The precision obtained from such methods is usually at least one order of magnitude greater than that necessary for quality control purposes. Most pharmaceutical products are dispensed in dosage forms which allow for a relatively large tolerance in the active ingredients/patient's body weight ratio or even in the amount dispensed. Thus for routine quality assurance methods, very high precision is not necessary. The present work reports some studies of the use of a rapid procedure for the determination of dosage forms of some antimalarial formulations.

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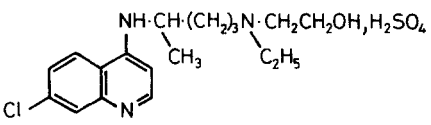
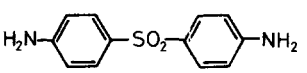
Malarial disease is still prevalent, especially in the tropics. The resistance of the malarial parasite to single drugs necessitates the need for multiple drug therapy. Thus various drug combinations have been employed. The advantages of using more than one compound combined in the dosage form is that it is possible that they will supplement each other, that the patient will take the full dose without omitting one of the parts or alter the drug ratios and it has been claimed that toxicity or patients' intolerance to a particular drug may be prevented. It is thought advisable to have all the components in one compact tablet. These combination tablets may contain the commercial excipients and active ingredients with 2 or 3 different physiologically functional groups which may require separate and sequential determinations. The success of such determinations often depends on the choice of solvent systems since the analytes are generally similar in overall chemical properties. Aqueous systems having a high dielectric constant do not have a significant differentiating effect on many individual acids or bases. Nonaqueous systems, which cause differences in the strengths, have been employed in an attempt to make use of the difference for the assay of compounds with physiologically and chemically similar groupings.

In the present work, glacial acetic acid has been the general solvent of choice because it increases the basicity of compounds such as amines due to a levelling/differentiating effect on some of these bases. It is well known that perchloric acid (titrant/catalyst) is a stronger acid in this solvent than in aqueous systems and is suitable for acid/base titrations of relatively weak basic substances. The theoretical basis for such reactions has been reviewed previously [1].

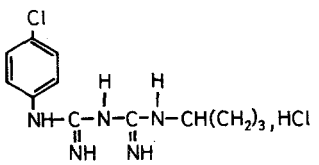
The use of solvent mixtures increases the number of systems available for nonaqueous titrations and hance the number of compounds that can be assayed. In the course of the present investigations, various solvents and mixtures were used to permit simple, separate and precise determinations of some drugs (structures I–IV) all of which have Lewis base properties (Fig. 1).



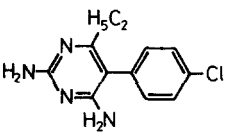
[I] Hydroxychloroquinesulphate



[II] Dapsone



[III] Proguanilhydrochloride



[IV] Pyrimethamine

Fig. 1

Although the thermometric techniques have been used for the analysis of organic compounds from 1910, no pharmacopoeia gives reference to them. Generally recommended techniques require the physiologically active analyte to be separated from the dosage form of the pharmaceutical product and many require assays to be done on some derivatised form of the analyte. Separations and derivatisation are to be avoided, if possible, since both losses and contamination may occur. It is considered by the present authors to be desirable to assay without prior separation of the analyte.

Previous work involving titrations with and without separation of the analyte has been reviewed by Grime [2]. Applications of the technique for dosage forms [3–7] and examination of the effects of various excipients [2–5] have been reported. Greenhow [5] advocates the use of the polymerisation of selected monomers as an indicator reaction to give better reproducibility.

The present investigation reports the use of the technique for the rapid and selective determination, without prior separation of the analyte, of the anti-malarial active ingredients of dosage forms of the commercially available tablets. The results are compared with those obtained from known amounts of the authentic drug substance, in the pure state, similarly treated.

Experimental

The apparatus used is, in principle, the same as that used by previous workers [8, 9]. The main difference is that the multi-roller peristaltic pump [8] or the motor driven Teflon syringe [9] was replaced by a motor-driven all glass syringe fitted with semi-flexible glass joints. This was required to prevent any reaction of the non-aqueous solvents and the reagents with the organic materials of the tubing previously used to give flexibility to the apparatus.

Recorder and syringe parameters

After preliminary experiments, these were adjusted so that the sensitivity of the recorder was sufficient to record the onset of the second (catalysed) reaction without giving significant shift for the preliminary analyte reaction. This also obviated electronic noise and gave acceptable smooth curves. The speed of the chart and the rate of delivery of the titrant were adjusted for each individual series of tablets so that the length of run was 20 nm for the standard amount of active ingredient stated by the manufacturers to be present in each particular series. (This allowed for easier routine comparison of the results from individual tablets in batches.]

Reagents and solutions

Perchloric acid. 71–73% v/v AnalaR reagent. Solutions of perchloric acid in acetic acid/acetic anhydride were standardised against a solution, prepared from A.R. potassium hydrogen phthalate which had been recrystallised and then dried to constant weight at 110°.

For sharp and better defined end points, the concentration of the titrant was arranged to be between 10–50 times the original concentration of the analyte.

Antimalarial Drug Substances: Chloroquine. Salts of Chloroquine. Dapsone. Pyrimethamine. Proguanil hydrochloride. (All supplied by commercial pharmaceutical companies.)

Tablets

Commercial name	Active ingredient	Nominal weight in tablet	Dosage amount
AVLOCLOR (I.C.I.)	Chloroquine phosphate	250 mg	300 mg weekly
NIVAQUINE (M&B)	Chloroquine sulphate	200 mg	400 mg weekly
PALUDRINE (I.C.I.)	Proguanil hydrochloride	100 mg	100–300 mg daily
PLAQUENIL (Winthrop)	Hydroxy chloroquine SO_4	200 mg	400 mg weekly
DARAPRIM (Wellcome)	Pyrimethamine	25 mg	25–50 mg weekly
MALOPRIM (Wellcome)	Dapsone	100 mg	1 tablet (112.5 mg)
	Pyrimethamine	12.5 mg	weekly

These were used as supplied by the manufacturers.

The approximate pH of an aqueous solution or suspension of each of the drug substances was about 7–8. They were therefore treated as weak organic bases.

The limit of solubility of each drug and reagent was tested in (i) water, (ii) glacial acetic acid, (iii) acetic anhydride, (iv) toluene, (v) 1,2 dichlorethane and (vi) nitromethane, to ensure that there would be no precipitation of the materials when mixing of solvents occurred during any titrations.

Solvents

Glacial acetic acid 99%: AnalaR reagent. Made anhydrous by adding acetic anhydride (1 : 12 v/v) and allowing the mixture to stand 24 hrs before use.

Acetic anhydride: Analytical Reagent Grade.

1,4 Dioxan: Distilled. Fraction from boiling range 100–102° used.

1,2 Dichlorethane: Distilled. Fraction from boiling range 82–85° used.

Nitroethane: Distilled. Fraction from boiling range 114–116° used.

Nitromethane: Distilled. Fraction from boiling range 100–101° used.
Toluene: Distilled. Fraction from boiling range 109–111° used.

Indicators

4-Hydroxy-4-methoxypentan-2-one/Acetic anhydride. (1 : 1 v/v)
Alpha-methylstyrene/Acetic anhydride. (4 : 1 v/v)
Dimethoxymethane/Acetic anhydride. (1 : 1 v/v)
1,3-Dioxalane/Acetic anhydride. (2 : 1 v/v)
These are laboratory grade except where used as solvents.

Solvent mixtures

In order to ascertain the optimum solvent system for each analyte, preliminary experiments were done with authentic samples followed by tablets similarly treated without prior separation of the physiologically active ingredients.

The following solvent mixtures were found to be acceptable for these series of assays:

Solvent (i) Acetic acid/Acetic anhydride. 12 : 1 v/v (HA/AA)
(ii) Nitromethane/Acetic anhydride. 4 : 1 v/v (NM/AA)
(iii) 1,4 Dioxan/Acetic anhydride. 4 : 1 v/v
(iv) Dimethoxymethane/Acetic anhydride. 4 : 1 v/v
(v) 1,2-Dichlorethane/Acetic anhydride 4 : 1 v/v

However, for the majority of the work the study was restricted to the first two systems.

Stoichiometry of analyte reactions

Weighed amounts of each active ingredient were titrated against standardised perchloric acid. From the weights taken and the volumes used, the stoichiometries of individual reactions were calculated. All the drugs except dapsone and chloroquine phosphate showed 1 : 1 reactions with perchloric acid. Dapsone and chloroquine phosphate showed 1 : 2 stoichiometries (i.e. one mole of sample to two moles of acid).

Effect of excipients

For each series of tablets, twenty tablets were powdered and dissolved in the appropriate solvent. Aliquots were taken and used in two sets of titrations. To one set was added a known amount of the authentic drug substance, the other set was used as prepared.

Each set was titrated with perchloric acid and the differences in the amounts of

perchloric acid used i.e. (amount determined experimentally − that obtained by calculation from the sum of the Volume use per aliquot + Volume required for the added drug) were noted.

In each case there was no significant difference (all differences were within experimental error). It was thus concluded that the excipients did not react with the perchloric acid solution.

Effect of indicators

Overall the effect of the various indicators used was to improve the sharpness of the stoichiometric end-point. All the indicators investigated, except alpha-methyl styrene, improve the sharpness of the end point when mixed with acetic anhydride in the ratio of 1 : 1 v/v or 1 : 2 v/v and about 1–2 ml of the mixture is used per 10 ml of the analyte solution. Alpha-methyl styrene polymerises in acetic anhydride when used in these ratios. The best ratio for alpha-methyl styrene/acetic anhydride mixture is about 4 : 1 v/v and about 2–3 ml of the mixture are used per 10 ml of the analyte solution.

The possible effects of having a large amount of indicator present were also investigated. It was found using known weights of the authentic drug samples, that increasing the amount of monomers (indicator) used in the titrations caused apparent premature end points. The amount of deviation from the true endpoint was fairly closely related to the amounts of indicator used.

It was generally found that the hydrolysis of the acetic anhydride was sufficient indication of the equivalence point for practical purposes.

For the above reasons, all further investigations were done without the use of the added monomers to act as indicators.

Precision and reproducibility

A series of 10 titrations was done for 10 mg amounts of each authentic drug substance as supplied by the manufacturers. The reproducibility was better than 1%.

10 separate tablets of each ethical preparation were assayed. The results are summarised in Tables 1 and 2.

Statistics

Tablet weights: Mean weights: 316.8 mg. Standard deviation: 6.72 mg. %Standard deviation: 2.12. Weight of analyte: Mean weight: 249.1 mg. Standard deviation: 1.68 mg. %Standard deviation: 0.67 (1%).

Table 1 Results for AVLOCLOR (Chloroquine phosphate)
Each tablet was stated by the manufacturer to contain 250 mg of active ingredient

Wt of Tablet (mg)	323.6	325.0	308.4	316.6	319.8	311.9	321.9	315.2	320.6	304.7
Wt found (mg)	247.7	250.0	250.0	250.0	248.7	250.0	247.4	250.0	247.5	250.0

Table 2 Similar results were recorded for each set of tablets

Tablet	(a)	(b)	(c)	(d)
Daraprim	31.76	0.56	25.0	0
Maloprim	142.92	2.5	112.5	0
Nivaquine	254.08	4.5	200.0	0
Paludrine	127.04	2.3	100.0	0
Plaquenil	254.08	4.5	200.0	0

(i) Mean weight (in mg) of individual tablets. (b) Standard deviation (in mg) of tablet weight. (c) Mean weight (in mg) of active ingredient per tablet. (d) Percentage Standard deviation, to the nearest 1%, of the amount of active ingeedient.

Time required for an assay used for quality assurance purposes

The time take for an assay can be divided into three sections:

(i) Time taken to dissolve the tablet, since weighing of the tablets is not necessary.

(ii) Time taken to titrate with a sufficient excess of titrant used to give a clear indication of the equivalence point.

(iii) Time taken to calculate the amount of analyte in the tablet. When the method is being used for quality assurance purposes, all that is required is to ascertain whether the amount of analyte lies outside the agreed limits.

The times taken to dissolve the tablets, with stirring, were usually of the order of 2–3 mins.

The rate of delivery of titrant and the concentration of the titrant are arranged so that for a standard amount of the analyte in the tablet, the end point is reached when 200 mm of chart has been used. This in practice requires a titration volume equivalent to about 230 mm of chart length. The chart speed is adjusted so that the deflection caused by the onset of the catalysed reaction is practically useful. Speeds of between 2 mm to 20 mm per second were used at various times. Thus the least amount of time for the titration is about 12 secs and the greatest time is about 2 mins.

Generally speeds of 10 or 20 mm per second were employed, viz.: times of titrations were about 12–25 secs.

All that is necessary to calculate the amount of analyte present is to measure the distance from the onset of the titration to the equivalence point (about 30 secs) and

compare this with the distance (200 mm) for a standard amount of analyte, the consult prepared calibration tables. For quality assurance purposes it is only required to see if the distance is within the agreed limits.

Thus, the time required to calculate the amount of material present was of the order of 1 minute. The total time for the assay of a tablet was of the order of 3–5 minutes.

Sequential determination of the active components of Maloprim

Since it is not possible to determine dapsone if acetic acid/acetic anhydride is used as the solvent, but it is possible to determine it, if the dapsone is dissolved in a nitromethane/acetic anhydride systems, it was though that the total basicity of the tablet mixture should be able to be determined if the solvent system is nitromethane (acetic anhydride and the pyrimethamine component should be the only compound determined if the solvent systems is acetic acid) acetic anhydride.

Thus, synthetic mixtures of the two drugs pyrimethamine and dapsone were made and titrated with perchloric acid in glacial acetic acid (for the mixture dissolved in an acetic acid/acetic anhydride system) and dissolved in nitromethane for the mixture dissolved in nitromethane/acetic anhydride system.

Weighed amounts of the mixture were stirred in contact with the acetic acid/acetic anhydride mixture for at least 5 minutes before being titrated with the perchloric acid in the same solvent.

Separate weighed amounts were dissolved in a nitromethane/acetic anhydride system and titrated with perchloric acid in the same solvent system.

The results are given in Table 3.

Table 3

Amount (in mM)						
Pyrimethamine	0	1	2	5	10	20
Dapsone	20	10	5	2	1	0
mM of $HClO_4$ used						
(in HA/AA)	0	1.01	2.0	5.0	10.02	20.01
(in NM/AA)	20	11.02	7.0	7.01	11.01	20.01

This affords a method of determining the two analytes: dapsone and pyrimethamine, which are used as a mixture in the compound Maloprim. The total basicity is determined using the tablets dissolved in nitromethane/acetic anhydride with the perchloric acid dissolved in the same solvent and then a separate titration is done with the tablet dissolved in acetic acid/acetic anhydride, allowed to stand at

room temperature for 5–6 mins and then titrated with perchloric acid dissolved in acetic acid/acetic anhydride.

Discussion

In any study involving solution thermochemistry used for quantitative purposes, the main concern is to obtain satisfactory indication of the equivalence points of all reactions. In any particular reaction system the quantity of heat change is constant and thus to obtain a sharp inflection the electronic sensitivity of both the bridge and the recorder can be adjusted. Similarly, the precision of the titration which is related to the length of chart used in the titration, which gives a measure of the amount of titrant delivered, is governed by the rate of addition of the titrant, the concentration of the titrant and the chart speed. Both sets of parameters are interdependent in determining the shape of the enthalpogram and thus the optimum set of conditions for each system is determined by trial and error.

A comparison of the thermograms obtained when the perchloric acid is dissolved in either the acetic acid/acetic anhydride or the nitromethane/acetic anhydride solvent systems, and the sample in either of the two solvents, shows that of the four possible combinations, although good results are generally obtained when the perchloric acid is dissolved in the system (acetic acid/acetic anhydride) containing the glacial acetic acid, this is not always the case and the optimum solvent systems should be found by experiment. It is found that the amount of acetic anhydride in the system needs to be determined by experiment. When nitromethane is used to dissolve the sample it is advisable to add some acetic anhydride to improve the indication of the end point. Tests were made to confirm that the presence of excess acetic anhydride is necessary to effect the catalytic action of acetacidium perchlorate at the equivalence point when the acetic anhydride reacts with the water present in the system.

Titrations were done in "glacial acetic acid for non-aqueous titrations" (obtained from B.D.H. and with a labelled water content of 0.01–0.2% w/w). No end point signal was observed but when 0.2–0.3 ml of acetic anhydride was added to the base solution, good end point indication was obtained. Thus the solvent must contain an excess of acetic anhydride to effect the catalytic action of the acetacidium perchlorate at the neutralisation point when the acetic anhydride reacts with the water present in the reaction system.

Considering the use of monomers as indicators for these thermal reactions, it is thought that although the kinetics of the analyte reactions are more favourable that the catalysed reactions, the relative amounts of analyte and monomer at or near the equivalence point may be such that the mass action effect means that the rate of the

reaction of the analyte compared to that of the catalysed reaction is small and the latter reaction becomes apparent before all the analyte has practically reacted. It is therefore considered to be better to avoid the use of separate indicator reactions in these systems.

It has been observed that although the choice of solvent system depends to some extent on the nature of the sample, dissolution of the sample in a particular solvent system does not ensure that the sample is titratable in that system; dapsone is not titratable if the acetic acid/acetic anhydride solvent is used. However, if the acetic acid is replaced with either nitromethane, toluene, 4-hydroxy, 4-methoxypentan-2-one or 1,2 dichlormethane; then the titration of dapsone is possible. Although this may be due to the levelling effects of the acetic anhydride/acetic acid mixture, it is thought that the use of the acetic acid/acetic anhydride solvent probably results in the relatively rapid acetylation of the functional groups of the analyte. This relatively rapid acetylation reaction does not occur with pyrimethamine, in the time of the titration and at room temperature. The primary amino groups are deactivated by the adjacent heterocyclic nitrogen atoms.

When dapsone is dissolved in nitromethane/acetic anhydride and titrated with perchloric acid in acetic anhydride, a satisfactory thermogram is obtained. To eliminate the heat of mixing and thence to effect possible enhancement of the equivalence point, the titration was repeated with both the dapsone and the perchloric acid dissolved in nitromethane/acetic anhydride. The thermogram was significantly improved. The reaction stoichiometry is one mole of dapsone to two moles of perchloric acid in each case.

The results of "spiking" tablets with the active ingredient indicated that the excipients used by the manufacturers when formulating the tablets have no significant effect on the method used to determine the amount of active ingredient present in the tablets. This is to be expected since it is common practice to include in formulations, which are used in an ingestible form, only those excipients which do not have any noticeable reaction with the constituents of the gut fluids. Essentially they are chemically inert towards the titration systems used in the above work. Separate tests made with excipients such as calcium lactate, talc, and small amounts of mould release agents such as stearates in previous investigations [5] have shown that acetic anhydride/perchloric acid is not consumed by these materials.

The percentage standard deviation for the assay of the active ingredients in the tablets is quoted to the nearest 1% since this is considered to be realistic in terms of quality assurance work.

The time taken for an assay should not be used in a direct calculation of the number of assays that can be done in a working day. Other factors such as whether or not the reaction vessel is re-used after being washed, hence requiring some time for the washing operation; if the tablets are weighed etc. all need to be considered. In

our work, we use low-cost, disposable plastic beakers which cost less to replace than the cost of having them washed, even by a non-skilled worker. The tablets do not require weighing for quality assurance or control purposes, the exact amount of analyte is not needed to be ascertained, it is only required to have an indication whether or not it is within the agreed commercial limits.

Conclusion

Investigations show that the method of solution thermochemistry has a part to play in the techniques used for quality control of some pharmaceuticals of antimalarial properties. There is no need to weigh the tablet/s which comprise the individual dosage forms of the drugs and it is not required to isolate the active ingredient, this combined with the use of semi-skilled labour able to follow simple instructions; the using of disposable reaction vessels; the necessity to use only administrational procedures which obviate calculations on the part of technicians, other than those trained in such skills, provides a very rapid and relatively low cost method for analysing dosage forms of the various drugs tested.

The individual analytes of a dosage form which contains two active ingredients can be assayed, if use is made of the differentiating effects of some non-aqueous solvents is possible.

The precision is within that required for many commercial purposes.

* * *

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Zusammenfassung – Es wird die Anwendung eines lösungsthermochemischen Verfahrens zu Schnellbestimmungen bei der Qualitätskontrolle und – sicherung von Wirkstoffeinsatzmengen einiger rezeptpflichtiger Präparate von einigen antimalarischen Arzneimitteln empfohlen. Die aktiven Bestandteile sind Chloroquin, Hydroxychloroquin, Dapson, Proguanilhydrochlorid und Pyrimethamin.

Die Bestimmungen werden ohne jegliche Abtrennung der Bindestoffe und ohne Isolierung oder Derivatisierung der zu bestimmenden Substanzen durchgeführt. Die Titrierungen werden in Eisessig durchgeführt und bedienen sich der katalytischen Hydrolyse von Essigsäureanhydrid durch Perchlorsäure als Endpunktindikation der Reaktion.

Die Dauer für eine typische Probe bei einer typischen Wirkstoffmenge beträgt etwa 3–5 Minuten. Die Reproduzierbarkeit liegt in der Größenordnung von 1% für Milligrammengen der zu analysierenden Stoffe im Arzneimittel.

Резюме — Предложено использование метода жидкостной термохимии для быстрого количественного анализа и количественного контроля дозируемых количеств некоторых антималярийных медикаментов. Активными компонентами были хлорокин, оксихлорокин, дапсон, прогуанилгидрохлорид и пириметамин. Анализ проводился без разделения компонентов и без выделения или без перевода анализируемых веществ в какое-либо их производное. Титрования проводились в среде ледяной уксусной кислоты и использовали каталитический гидролиз хлорной кислотой уксусного ангидрида для обнаружения конечной точки реакции. Время анализа типичной дозы препарата составляло 3–5 минут. Воспроизводимость анализа составляла 1% для милиграммовых количеств анализируемого вещества, присутствующего в дозируемом препарате.