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Design of a dissolution system for the evaluation of the release rate characteristics of artemether and dihydroartemisinin from tablets

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Abstract

As none of the pharmacopoeial dissolution methods are suitable to evaluate the release rate of artemether and dihydroartemisinin from tablets, a 'two-phase partition-dissolution' method, based on the one of [J. Pharm. Sci. 85 (1996) 1060] was developed. It consists of an organic solvent in the upper part and the aqueous phase, in which the dissolution test was executed. The main requirements for the selection of the solvent are: the density should be lower than 1; the analyte should dissolve in the organic part as much as required for 'sink' conditions; if possible, the cut off should be near 200 nm, which allows direct HPLC measurement at 215 nm. The most suitable solvent for artemether is isooctane in a ratio of 100/150 ml aqueous phase. Samples could be analysed without further treatment. For dihydroartemisinin, chlorobutane was selected in a ratio 150/150 ml water. In the latter method, the solvent disturbed in the HPLC analysis and therefore samples were evaporated and then reconstituted in methanol. Repeatability of the test was satisfactory and discrimination ability tests on Artenam® tablet batches and self-made dihydroartemisinin tablets, respectively, showed good results, confirmed via calculation of the similarity factor f_2 (value <50). Dissolution determination of Cotecxin® tablets was proven not to be conform as immediate-release tablet.

Keywords: Two-phase partition-dissolution; Dihydroartemisinin; Artemether

1. Introduction

The recent and widespread appearance of counterfeit antimalarial tablets prompted the search for simple evaluation techniques to identify and quantify the active in pharmaceutical formulations (Green et al., 2001). The poor quality of drugs has been linked to counterfeiting of medicines, chemical instability especially in tropical climates and poor quality control during manufacturing (Taylor et al., 2001). These conditions can influence the bioavailability of the drug from the tablet. One should pay attention to it, as widespread use of such medication is probably the cause of mortality and morbidity due to malaria (Rozendaal, 2001; Newton et al., 2001). Moreover, such preparations could lead to therapeutic failure (Taylor et al., 2001) and increase the development of drug-resistant organisms. For tablets, the pharmaceutical technological quality, especially the dissolution profile should be evaluated. The in vitro tests allow us for instance to evaluate the release rate properties

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of the active compound; it assesses the batch-to-batch quality in industrial production, expressing changes in formulation, manufacturing process and so on.

Several formulations based on artemisinin derivatives, especially artesunate, artemether and dihydroartemisinin are already available in many countries but their use is poorly regulated (White et al., 1999). First of all, pharmaceutical quality of these products should be evaluated; especially in tablet evaluation, a dissolution test is required. The main problem of these products is their poor solubility in water, and so the maintenance of sink conditions should be well studied. Under these conditions, the drug concentration in the dissolution medium should not exceed 10 or 20% of its solubility in the medium (Posti and Speiser, 1980).

Different methods are described in literature to overcome problems concerning maintenance of the sink conditions: high and fixed volume dissolution media; continuous flow (flow-through cell); addition of solubility enhancers such as sodium laurylsulfate as it is allowed in the US Pharmacopoeia (Shah et al., 1989); co-solvents in dissolution media; multiple phase systems, which allows dissolution process of the drug in the aqueous phase, followed with an extraction to an organic phase, in which the analyte is well soluble. The method of Hoa and Kinget (1996), developed for obtaining the dissolution profile of artemisinin in tablets, is based on that.

The purpose of this work was to evaluate the presented methods and their applicability in the determination of dissolution profiles of artemether and dihydroartemisinin in tablets. The criteria for the dissolution test and instrumentation used were followed as described in the European pharmacopoeia. In contrast, the limits for release of the drug in a certain time span for an immediate release tablet are not clearly stated in the different pharmacopoeia. The USP comprises several monographs of tablets, containing a specific active compound. The dissolution test described for these formulations contain specific criteria concerning dissolution medium, paddle or basket method and so on. For example the dissolution profile of furosemide tablets should be evaluated using the paddle method at 50 rpm in a phosphate buffer pH 5.8. The USP requirement is that percent drug release at 60 min is not less than 85% (USP 24, 2000; Qureshi, 1996).

The “Guidance for Industry” (US Department, 1997), developed by the FDA, follows the criteria of the USP, but adds practical detailed description for the performance of the dissolution test. In general mild agitation conditions should be maintained during dissolution test to allow maximum discriminating power and to detect products with poor in vivo performance. Using the basket method, the common stirring speed is 50–100 rpm with the basket method and 50–75 rpm with the paddle method. The volume of the dissolution medium is generally 500, 900 or 1000 ml. Sink conditions are desirable but not mandatory. An aqueous medium with a pH range 1.2–6.8 should be used. For water insoluble or sparingly water soluble drug products, use of a surfactant such as sodium lauryl sulfate is recommended. The need for and the amount should be justified. Use of a hydroalcoholic medium is discouraged (EMEA, 2001).

The “Fédération International Pharmaceutique” defined an immediate release tablet, in which 75% drug release occurs within 15 min (Aiache et al., 1996). In this case, single point dissolution data are sufficient to evaluate the dissolution characteristics of the tablet. Immediate-release formulations with a specified dissolution time of more than 15 min will require an in vitro–in vivo comparison study and dissolution profiles based on several points (at least 3). At each dissolution point, the difference of measurements of six tablets should not vary more than 20% unless clinical reuses have been shown to provide reproducible and acceptable in vivo performance.

The recommendations of the EMEA include the measurement of at least three points to determine the dissolution profile, especially for tablets with sustained release characteristics (EMEA 1999a, b): the first one at 20–30% release of the drug to detect dose dumping: the second one, determining the shape of the dissolution curve, namely at 50% release; and one point, showing the release of the great fraction of the drug from the tablet namely at 80%. Variation in dissolution results of different batches should be proven acceptable via in vivo testing. Normal inter-batch-variability of 10% is accepted. Tolerance of greater batch variations should be proven via bioequivalence studies.

At the time being, the little advice given by the discussed pharmacopoeia seemed to deviate much from each other (Aiache et al., 1996). As USP defines

clearly acceptance criteria for immediate release tablets, the tablets investigated in this work, will be evaluated for their conformity to these criteria.

2. Materials and methods

2.1. Materials

As standard products, β-artemether and dihydroartemisinin, kindly received from Arenco Pharmaceutica (Geel, Belgium), were used. For analysis on HPLC, acetonitrile HiPerSolv® for HPLC from BDH Laboratory Supplies (Poole, UK), potassium hydrogenophosphate (extra pure crystals) from Merck (Darmstadt, Germany) were used. Methanol or acetonitrile HiPerSolv® for HPLC from BDH Laboratory Supplies were used in sample and standard preparation. The following dissolution media were evaluated: MQ water and 0.1N HCl were used in the high volume dissolution test and flow-through method; organic solvents were employed in the two-phase partition-dissolution method namely, cyclohexane Lichrosolv®, chlorobutane pro analysi, isooetane pro analysi, *n*-hexane Lichrosolv®, petroleumether 50–70 °C pro analysi, methyl-*tert*-butylether pro analysi, all purchased from Merck (Darmstadt, Germany) and *n*-butanol UV–IR–HPLC from Panreac Quimica SA (Mont Cada, Spain).

2.2. Tablet preparations

2.2.1. Tablets formulations available on the market

For the development and evaluation of the dissolution methods, two tablets, containing artemether, and one, containing dihydroartemisinin, were used: Artenam®, containing 50 mg artemether per unit of 200 mg (Arenco Pharmaceutica, Geel, Belgium); Coartem®/Riamet®, containing 20 mg artemether and 120 mg lumefantrine (Novartis Pharma, Basel, Switzerland) per unit; Cotecxin® containing 60 mg dihydroartemisinin per unit (Bejing Cotec Bejing Sixt Pharmaceutical Factory, Bejing, China). For all these formulations, the conformity with the European pharmacopoeia was evaluated concerning their weight variation, disintegration, friability and content uniformity. Results are further presented in Table 1.

2.2.2. Self-made tablets

To study the ability of the method to discriminate similar tablets, having a different crushing strength, namely 30, 50 and 140 N, tablets were prepared with the following composition:

Dihydroartemisinin 50 mg, lactose (DMV international, Veghel, The Netherlands) 116.2 mg, Crosscarmellose sodium NF (FMC Corporation, Brussels, Belgium) 2 mg, microcrystalline cellulose (FMC Corporation, Brussels, Belgium) 30 mg, silicium dioxide (Federa, Brussels, Belgium) 0.4 mg and magnesium stearate (Federa, Brussels, Belgium) 1.4 mg.

The wet granulation technique was applied. All ingredients except for magnesium stearate and Aerosil® 200, were mixed in a PVC box for 5 min in a Turbula mixer model T2A from W.A. Bachofen Maschinenfabrik (Basel, Switzerland). A small amount of water was added to wet the powder blend. The latter was then granulated in a granulating apparatus Erweka AR 400 (Heusenstamm, Germany) and subsequently dried at room temperature. The whole mixture was then passed through a 0.8 mm sieve from Erweka (Heusenstamm, Germany). Aerosil® 200 was also sieved through a 0.8 mm sieve and added to the tablet mixture. Then, the mixture was again mixed during 1 min. The same procedure was followed for magnesium stearate without the sieving step. The tablets were compressed in a single punch-tabletting machine Baby S-728 from Courtoy N.V. (Halle, Belgium) using a Piezo electric cell, measuring the compression force.

2.3. Evaluation of tablets

In addition to the dissolution test (see paragraph 2.7), the following tests applied in the evaluation of tablets, are:

- *Weight uniformity of the tablet*: The uniformity of tablet weight was investigated. Following the instructions of the Eur. Pharm. IV, 20 random selected units were weighed separately on a Sartorius Analytic type A 200 S balance and the variation coefficient or relative standard deviation (RSD) of the tablet weight was calculated;
- *Crushing strength*: The DHA tablets were compressed to a determined crushing strength by

Table 1
Results of validation tests on the different artemether/dihydroartemisinin tablets, used in the dissolution development

	Mean	VC (%)	Minimum–maximum
Artenam® batch 1			
Mean weight of tablets (mg)	203.1	1.52	198.1–208.2
Content (%)	103.04	2.27	99.0–104.6
Disintegration time (min)	3.43	15.24	2.67–4.17
Crushing strength (N)	96.0	9.81	85.0–112.0
Friability (%)	0.15	–	–
Artenam® batch 2			
Mean weight of tablets (mg)	197.6	0.98	193.5–201.0
Content (%)	96.4	2.02	95.0–99.3
Disintegration time	–	–	–
Crushing strength (N)	66	8.73	54–76
Friability (%)	–	–	–
Coartem®			
Mean weight of tablets (mg)	243.0	1.03	239.0–248.0
Content (%)	97.5	2.18	96.0–99.0
Disintegration time (min)	3.62	9.95	3.15–4.11
Crushing strength (N)	102.5	13.20	76.0–120.0
Friability (%)	0.18	–	–
DHA tablets batch of low crushing strength (around 35 N)*			
Mean weight of tablets (mg)	205.5	0.98	202.1–208.6
Disintegration (s)	<20	–	–
Crushing strength) (N)	37.1	17.53	30–50
Friability (%)	2.97	–	–
DHA tablets batch of medium crushing strength (around 100 N)*			
Mean weight of tablets (mg)	214.4	0.59	210.2–213.7
Disintegration time (min)	2.09	17.07	1.63–2.67
Crushing strength (N)	103.75	16.87	82–128
Friability (%	1.90	–	–
DHA tablets batch of high crushing strength (around 150 N)*			
Mean weight of tablets (mg)	215.9	1.74	209.5–220.18
Disintegration time (min)	21.57	13.83	18.2–25.73
Crushing strength (N)	159.3	4.50	143.0–166.0
Friability (%)	0.65	–	–

* Dosage, performed on the tablet blend: 53.35 mg per tablet.

varying the compression force. Ten tablets of each batch of as well as the commercial as the self-made tablets were subjected to the test measuring the strength in Newton, using a Heberlein Schleuniger instrument (model 2E, Dr. K. Schleuniger, Zürich, Switzerland);

- *Friability*: As prescribed by the Eur. Pharm. IV, the friability (Fr) was determined in a Roche friabilator from Erweka (Heusenstamm, Germany) as follows. Ten tablet units with a known total weight were turning in the apparatus at 25 rotations per minute for 4 min. The total weight of the tablets after the test was determined. The Fr (%) was calculated. Acceptable values are limited to 1%;
- *Determination of the disintegration time*: Following the general requirements of the Eur. Pharm. IV the disintegration test was performed on six tablet units in 900 ml water at 37 °C.
- *Determination of the content of the active compound per tablet*: The dosage per unit was also evaluated, if enough sample was available. Therefore, the tablet was dissolved in an appropriate amount of methanol, as such that a solution of artemether around 0.5 mg/ml was obtained. If necessary, the

solution was then centrifuged or filtered and then subject to HPLC analysis.

2.4. Sample treatment

2.4.1. Sample reconstitution

If the solvent of the samples does not interfere with the analysis of artemether or dihydroartemisinin, they can be subjected directly to HPLC analysis. If interference was observed, a certain amount of the samples was dried under N_2-stream and reconstituted in the same volume with methanol.

2.4.2. Sample preconcentration

If required, aqueous 'dissolution' samples of artemether were preconcentrated as follows: 15.0 ml sample was extracted with 10.0 ml isooctane. Samples were shaken during 15 min. A volume of 5.0 ml was dried under nitrogen stream and reconstituted in an appropriate volume of methanol, depending on the factor of preconcentration. The samples were then filtered through a Millipore Optex filter 0.2 μm from Omega Pharma (Nazareth, Belgium) before injection into the HPLC.

2.5. HPLC analysis

Starting from a stock solution of 100 mg/100 ml artemether and dihydroartemisinin in methanol, five dilutions were prepared in the range of 20–100 mg/100 ml in the same solvent. Every day of analysis, some of the standards were analysed on HPLC and the dosage determination of the samples was then based on these results.

Artemether and dihydroartemisinin standard solutions and samples ranging from 0.1 to 1 mg/ml were chromatographed on a CC Nucleosil® column-120 mm-5 C_{18} Chrom-Cart-HPLC-Trennsaüle from Machery & Nagel (Düren, Germany). The mobile phase for both compounds consists of 0.05 M KH_2PO_4 in water/acetonitrile/water in a ratio of 320/480/200 (v/v/v) for dihydroatemisinin and 320/480/100 (v/v/v) for artemether. The flow rate was always 1 ml/min. The liquid chromatograph consists of a Binary LC-250 pump, a Diode array LC 235 detector installed at 215 nm and an LCI-l00 integrator. Injection volume is always 20 μl by use of a loop from Rheodyne (Berkeley, USA).

2.6. Solubility determination

The solubility of β-artemether and dihydroartemisinin in organic solvents has been determined semi-quantitatively or quantitatively, depending on the level of solubility, by adding small amounts of the analyte in 25 ml of the solvent up to precipitation. The solubility of the analyte was determined quantitatively on HPLC after centrifugation with a Mistral centrifuge at 2000 rpm.

2.7. Description of apparatus and general procedures for dissolution studies

Drug release was measured using two dissolution apparatus, described in the Eur. Pharm. IV: the paddle apparatus for the high volume, the two-phase partition-dissolution and the cosolvent method and then, the flow-through cell for the flow-through dissolution method.

The first apparatus consisting of six recipients in a warm water bath at 37 °C, was purchased from Hanson Research (California, USA); the flow-through dissolution apparatus (Erweka Instruments, Heusenstamm, Germany), fitted with one cell for the dissolution of tablets.

In the 'high-volume dissolution' test, the volume was kept at 1000 ml. In the 'cosolvent' method, a solution of methanol/water at a ratio of 3:1 (v/v) was tested.

For the two-phase partition-dissolution, the best organic solvent should be determined. The dissolution volumes were selected as such that the paddle, was positioned at the interface of the two liquids in the system. The ratio organic/aqueous phase is *x* ml/150 ml, depending on the analyte.

At predetermined time intervals, samples of 5 or 10 ml were taken and subjected to HPLC analysis, directly or after sample treatment. With the paddle method, sample volumes were replaced by fresh dissolution media. The rotation speed was kept at 50 or 100 rpm, depending on the test. The selection of the dissolution medium will be investigated.

In the flow-through method, the dissolution medium enters the cone through a capillary bore on the bottom and flows up in the cell. The lower cone was partially filled with glass beads, which serve to equalize the jet of fluid entering the cell (Bhattachar

et al., 2002). The flow rate in that system was 16.7 ml/min.

2.8. Dissolution calculations

For the determination of the amount active compound released at a determined time point, the following formula was considered for the 'high volume dissolution' method and the 'two-phase partition-dissolution' method to calculate the cumulative amount of drug released at time t_i:

$$\text{mg dissolved } t_i = \frac{PA_{Sa}}{PA_{St}} x\ f\ x\ C_{St}\ x\ V_v + \sum_{x=2}^{x=i-1} \frac{PA_{Sa}}{PA_{St}} x\ f\ x\ C_{St}\ x\ V_S$$

where PA_{Sa} is the peak area of the sample on HPLC at 215 nm; PA_{St} is the peak area of the standard on HPLC at 215 nm; C_{St} is the concentration of the standard (mg/l00 ml); V_v is the volume of the dissolution medium (in the paddle method) or the organic phase (in the two-phase partition-dissolution method) from which the sample was taken; f is the preconcentration factor, if applied; V_S is the sample volume taken at time t_1 (ml).

The calculations in the flow-through cell-method were based on the formula for the determination of the area of a trapezoid.

The area of the trapezoid was calculated between two points of sample taking, which is characteristic for the amount of artemether released between two time intervals. The trapezoid is the area under the straight line for which the area is calculated as follows:

$$A_i = \tfrac{1}{2}(V_b - V_a)(y_0 + y_1)$$

where A_i is the surface of the trapezoid between two time points a and b; V_a is the volume of dissolution medium passed through the cell at time point a (ml); V_b is the volume of dissolution medium passed through the cell at time point b (ml); y_0 is the concentration of artemether in the sample at time point a (mg/100 ml); y_1 is the concentration of artemether in the sample at time point b (mg/100 ml).

At each time point, the amount of drug released was determined as the sum of area at that time point and previous area of the trapezoid.

2.9. Evaluation of the similarity of two sets of tablets: comparison of dissolution profiles

The US Pharmacopoeia sampling acceptance dissolution criteria constitute the simplest approach to compare dissolution data, defined as a minimum, a maximum or a range of percentage dissolved mean values at specific time points.Additionally, the following factor is recommended by the USP:

$$f_2 = 50 \log \left\{ \left[1 + \frac{1}{n} \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\}$$

where R_i and T_i are the cumulative percentage dissolved of the reference and test profile, respectively at each of the selected ith time points.

The factor f_2 is inversely proportional to the average squared difference between two profiles, which emphases on a larger difference among all the time-points. It measures the closeness between the two profiles. The criteria to which the data to evaluate, should be conform, are: to use mean data, the percent coefficient of variation at the earlier points should preferably not be more than 20% and at the other time points should not be more than 10%; the dissolution measurements of the two products should be made, e.g., for immediate release products at 15, 30, 45, 60 min; because the f_2 values are sensitive to the number of dissolution time points, only one measurement should be considered after 85% dissolution of the product; for products which are rapidly dissolving, i.e., more than 85% in 15 min or less, a profile comparison is not necessary; an f_2 value in the range of 50–100 ensures sameness or equivalence of the two curves.

3. Results and discussion

3.1. Calibration line

All measurements for artemether and dihydroartemisinin samples were performed on HPLC at 215 nm. The equation obtained for artemether was: $y^* = 29200x^* + 1755.2$ ($r = 0.9999$). For dihydrorartemisinin, value of the peak area was determined as the sum of the peak area of the α-isomer ($t_R = 2.94$ min) and the β-isomer ($t_R = 4.23$ min).

The equation line obtained for the calibration is $y^* = 30110\,x^* + 162794$ ($r = 0.995$)(y^* = peak area; x^* = concentration of artemether (dihydroartemisinin) in solution, expressed in mg/100 ml).

3.2. Selection of the dissolution medium

Generally, the Pharmacopoeia recommends 0.1N HCl as dissolution medium. To evaluate stability and solubility of artemether and dihydroarternisinin, over-saturated solutions were performed at 25 °C in water and HCI 0.1N and the supernatant was investigated. The solution was injected on HPLC and a spectrum between 200 and 400 nm was taken.

From the results on HPLC and UV, the following could be observed.

Artemether as well as dihydroartemisinin seemed to degrade in 0.1N HCl already after one hour. UV spectra confirmed the degradation into a UV-detectable compound, having a maximum at 250 nm. In literature, several studies are published proving the conversion of artemisinin compounds into UV-detectable products when, subjected to acidic solutions. Thomas et al. (1992) suggested that artemether degrades within 15 min and dihydroartemisinin within 45 min, when subjected to 5 M HCl. Zhao and Zeng (1985) showed that artemisinin was converted into Q_{290}-compound, having a maximal wavelength at 290 nm, after reaction with sodium hydroxide; subsequent addition of HCl led to the formation of a Q_{260}-compound with a maximal wavelength at 260 nm. Despite other conditions of temperature, than those, used by Zhao and Zeng (1985), a similar reaction within one hour at 25 °C seemed to occur in our selected dissolution medium, which is not acceptable for our purpose.

Due to degradation, it is clear that 0.1N HCI is not a suitable dissolution medium for the two actives. Water was therefore selected as dissolution medium and the solubility of both compounds was determined. Similar to previous tests, saturated water solutions were analyzed, giving the following results. The solubility of artemether and dihydroartemisinin in water was respectively 16.12 mg/100 ml ($n = 3$) and 15.14 mg/100 ml ($n = 2$) at 25 °C and no degradation could be noticed even after one week of storage.

3.3. Selection of the pharmaceutical formulations for the evaluation of the dissolution methods

Three tablets on the market, containing artemether or dihydroartemisinin, were investigated (Table 1). To test the discrimination ability of the final dissolution method, tablets of dihydroartemisinin with different crushing strengths and friability were prepared and subjected to the dissolution test.

To ensure the quality of these products and the self-made tablets, they were subjected to the tests, as mentioned in Section 2.3. The results are given in Table 1.

For all tablets, content, mean weight and disintegration time are conform with the requirements of the Pharmacopoeia, except for friability of the dihydroartemisinin tablets with low and medium crushing strength.

3.4. Evaluation of a 'high volume dissolution' method

To allow measurement of samples on HPLC at 215 nm without any pretreatment (measurable range: 1 till 100 mg/100 ml), the volume of the dissolution medium for tablets containing 50 mg active compound should not exceed 200 ml. However, the requirements of 'sink' conditions are then not fulfilled.

Dissolution of Artenam® tablets (50 mg AM) and Coartem® tablets (20 mg AM) was therefore performed in 1000 ml dissolution medium, followed by a preconcentration procedure (see Section 2.4.2). Results of these tests are presented in Fig. 1.

Based on these data (Fig. 1), the method seemed not that suitable. Despite of the fact that the sink conditions are more or less maintained, the maximum released content of artemether from the investigated tablets is at least three times lower than the solubility found at 25 °C, only 40% (20 mg) AM from the Artenam® tablet and 70% (15 mg) were released from the Coartem® tablet after at least 4 h of dissolution For the last one, simultaneous dissolution of lumefantrine from the Coartem® tablet can compete the release of artemether. Although good tablet characteristics of both formulations were observed (Table 1), great variations of percent release per time point were noticed, especially in the profile of the Coartem® tablet (Fig. 1). The used extraction technique for the preconcentration does not simplify

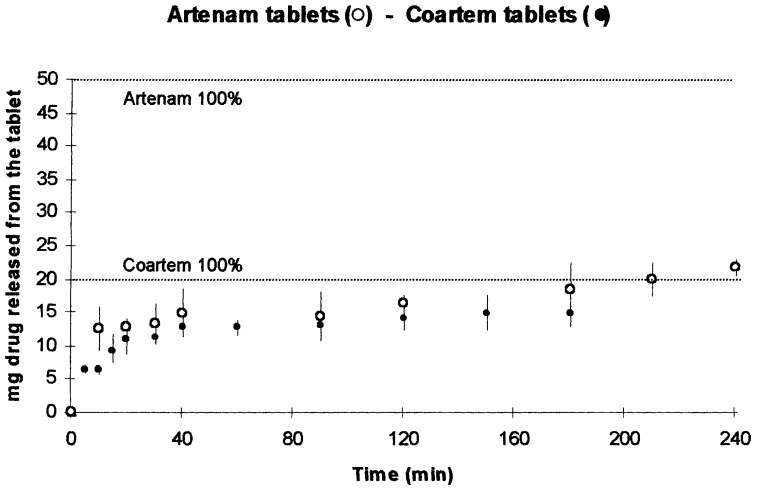

Fig. 1. Dissolution profile of Artenam® tablets (50 mg AM) and Coartem® tablets (20 mg AM) in 1000 ml water ($n = 2$).

the procedure. The system seems to be not acceptable.

3.5. Evaluation of dissolution methods using 'cosolvents' in the dissolution medium

Methanol/water (75/25) (v/v) was selected for application in the cosolvent method. From solubility tests of artemether in that solvent mixture (48.9 mg/100 ml), we could conclude that this method seems not more convenient than previous method. At least 300 ml dissolution medium should be used to maintain sink conditions in the dissolution method, applied on a tablet containing 50 mg artemether. An extraction technique to preconcentrate the sample is therefore required.

3.6. Evaluation of 'flow-through cell dissolution' method on artemether tablets

The method, recommended by the European and USP pharmacopoeia, for poorly water soluble drugs, is the 'flow-trough cell' dissolution method. Advantages of such method are well described in literature. Most important for our purpose is that the sink conditions should be maintained. The samples of the flow-through dissolution test on Artenam® tablets (50 mg AM) were preconcentrated, using the method discussed in Section 2.4. Calculations are performed based on the formula discussed in paragraph 2.8. Results are presented in Fig. 2.

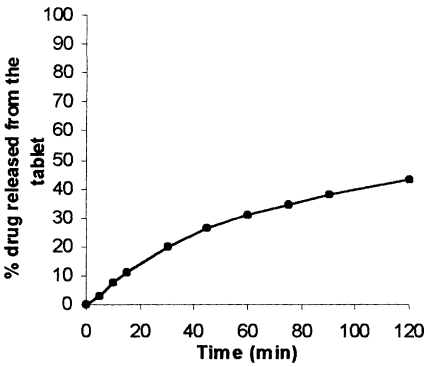

Fig. 2. Dissolution profile of Artenam® tablets with the 'flow-through dissolution' method ($n = 1$).

A much better dissolution curve compared to the 'high volume dissolution' method was obtained but the release rate of artemether from the tablet is very slow. Only 40% (20 mg AM) is released within 2 h.

At the earlier points, the determined concentrations were only three times lower than the one in saturated conditions. Therefore, we can conclude that the sink conditions were not maintained during the test, which is comparable to the results of Hoa et al. (1996) on artemisinin capsules at a flow of 1 l/h. Moreover, it is a time-consuming method due to the required preconcentration step, and is therefore not further investigated. The pre-concentration steps make the method laborious.

3.7. Evaluation of a 'two-phase partition-dissolution' method on artemether and dihydroartemisinin tablets

3.7.1. Selection of the suitable solvent for artemether and dihydroartemisinin in the 'two-phase partition-dissolution' method

Considering the work of Hoa and Kinget (1996), the possibility to develop a similar method, is investigated. In such system, the dissolution is performed in the aqueous phase; a continuous extraction of the drug into an organic phase is performed to maintain sink conditions in the dissolution part. This test is performed in the normal dissolution apparatus as described in the Eur. Pharm. IV.

Hoa and Kinget (1996) developed a 'two-phase partition-dissolution' system for artemisinin, using water and chloroform, in which the organic phase comprises the lower part. Therefore, a special tablet holder was constructed, consisting of a glass cylinder with a filter on the bottom, to avoid tablets pieces leaving the holder. Sample taking is performed by passing through the aqueous phase. A special mixing blade was fixed on the paddle to ensure the mixing of the aqueous phase and the dissolution of the drug in the special recipient. Measurement of the sample was performed with UV spectrophotometry after derivatization. In contrast with the method of Hoa and Kinget (1996), the system presented here uses the normal position of the paddle, as prescribed by the Eur. Pharm. IV. The most important advantage is that the dissolution of the tablet is performed on the bottom of the dissolution vessel. The sample is taken without passing through the aqueous phase. Consequently, the upper part in the system should consist of an organic phase, having a density lower than 1. The volume of the organic phase should be kept as low as possible allowing direct measurement at HPLC.

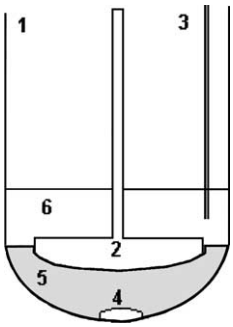

Fig. 3. Dissolution apparatus in which the 'two-phase partition-dissolution' is performed, showing the conditions of volume and partition in (1) the dissolution vessel; (2) paddle; (3) pipet for manual sampling; (4) tablet; (5) aqueous phase (150 ml); (6) organic phase (100 or 150 ml depending on the drug to analyze).

Therefore, solvents which could he used, were considered concerning their boiling point, density and cut-off. Table 2 represents solvents and their characteristics, which are evaluated for their suitability in the 'two-phase partition-dissolution' method.

Other criteria for selection of solvents to the density are: the boiling point should be higher than 37 °C and the cut-off to allow direct HPLC-measurement at 215 nm. Except for iso-pentane, all solvents were suitable for further investigation.

To maintain 'sink' conditions, the solubility of artemether should be at least 500 mg in the volume of the organic phase for a tablet with a dose of 50 mg.

Therefore, the following system was set up (Fig. 3). To lit the paddle in the middle of the separation front between the aqueous phase (in the lower part) and

Table 2
Evaluation of the solvents, based on their density, miscibility with water, boiling point, and cut-off, evaluated for their suitability in the 'two phase-partition dissolution' method

Solvent	Density (g/ml)	Miscibility with water (g/l)	Boiling point (°C)	Cut-off (nm)
Cyclohexane	0.766	–	80.7	200
Chlorobutane	0.885	1.1	78.0	220
Isooctane	0.701	–	99.3	197
n-Butanol	0.800	77	99.5	210
n-Hexane	0.660	–	69.0	195
Petroleumether 50–70 °C	0.665	–	50–70	200
Isopentane	0.618	0.36	27.8	195
Methyl-*tert*-butylether	0.742	51	55.2	210

Table 3
Solubility of artemether and dihyodroartemisinin in different organic solvents

Solvent	Artemether (mg/ml)	Dihydroartemisinin (mg/ml)
Cyclohexane	>60	–
Chlorobutane	>60	3.3
Isooctane	>60	<2.1
n-Butanol	>60	–
n-Hexane	>60	<1.2
Petroleumether 50–70 °C	>60	<1.4
Methyl-*tert*-butylether	>60	–

the organic phase (in the upper part), the volume of aqueous phase should be 150 ml. To obtain samples, directly measurable on HPLC, the volume of the organic phase should be as low as possible but at least 100 ml to have space for sampling. Solubility tests for artemether revealed that all solvents (Table 3) were suitable for artemether as in all investigated solvents solubility is higher than 600 mg/100 ml, thus more than ten times higher than the maximal release of drug from the presented tablet. The volume of the organic phase in the 'artemether' system can be fixed at 100 ml. other criteria such as interferences in the analysis should he investigated to find the most suitable solvent. In contrast, for dihydroartemisinin, only chiorobutane seemed to be suitable as organic phase in the dissolution system. But even with this volume (100 ml), the sink condition are not obtained. Therefore, the organic phase was set on 150 ml, which can dissolve almost 500 mg dihydroartemisinin.

3.7.2. Optimization of the conditions for the 'two-phase partition-dissolution' procedure for artemether tablets

Before starting the dissolution test, the behaviour of each organic solvent (in a ratio of 1/1.5 v/v with water) in the two-phase partition-dissolution system at 37 °C was evaluated. A clear distinctive solvent front should be obtained. Except for *n*-butanol, all solvents have a clearly separated front between both phases. Possibly due to the fact that 77 g *n*-butanol can dissolve in 1 l water at room temperature, both phases of butanol/water in that ratio are miscible at 37 °C. Therefore, *n*-butanol is rejected.

Another criterion is the interference on HPLC analysis. To analyse the samples directly on HPLC without any sample pretreatment, interference of the solvent with the artemether peak should not occur. When injecting solution of artemether, dissolved in methyl-*tert*-butylether, the peak of artemether seemed to be flattened. For that reason, this solvent was also rejected for further investigation.

Chlorobutane was rejected for further investigation as the plastic dissolution vessel was damaged by the solvent chlorobutane.

To select further the best organic solvent, e.g., *n*-hexane, isooctane or petroleumether, dissolution profiles of artemether from Artenam® tablets from the same batch, were performed on two samples in 150 ml water/100 ml organic phase, following the other conditions as discussed in Section 2.7. The rotation speed of the paddle is 100 rpm. Results are given in Fig. 4.

Based on these dissolution profiles, isooctane showed a regular dissolution profile, but some variation of around 20% on the sample at 30 min was observed, possibly caused by differences in the tablet hardness. Moreover, compared to the results of isooctane and hexane, the dissolution of artemether from the Artenam® tablet is much retarded, when using petroleumether.

As well as for hexane (53.26 and 53.61 mg artemether per unit) as for petroleumether (51.03 and 55.36 mg artemether per unit), higher doses exceeding the dose range of the tablet (47.5 –52.5 mg per unit), were found.

Based on that, the following could exist: a positive error on the extraction of artemether into the organic phase or the last one is evaporating slightly. The boiling points of hexane and petroleumether (see Table 2) are much lower than for isooctane.

Isooctane was selected as is the most suitable extraction solvent. Repeatability and discriminating abilities of different tablet batches will prove the suitability of the method: two batches were subjected to the presented dissolution test namely the 'two-phase partition-dissolution' test. The results of six samples are presented in Fig. 5.

It is obvious, from the results presented in Fig. 5, that the first batch seemed quite well different from the second one. The release is much lower; it took an hour to release 80% active compound of the first batch compared to the same amount within 15 min for the second one. A great intra-batch variation in the first

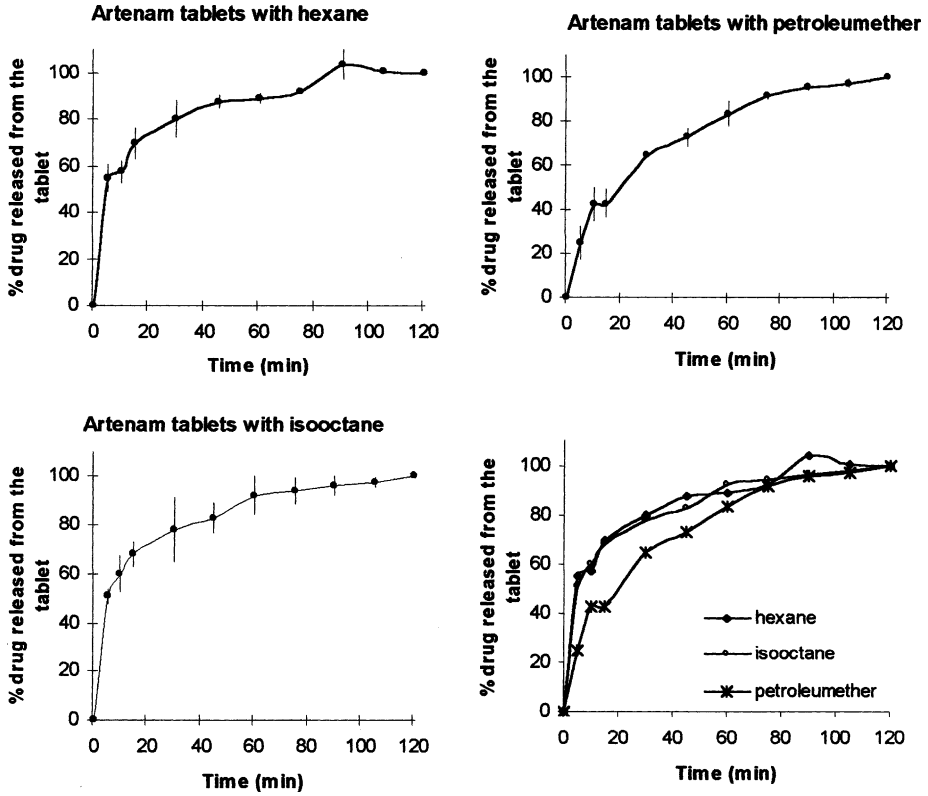

Fig. 4. Dissolution profile of Artenam® tablets with the 'two-phase partition-dissolution' method, using *n*-hexane, petroleumether and isooctane as extraction medium.

one is also noticed. When verifying the conformity of the batch at each time point according to the criteria of the USP (USP 24, 2000), batch 1 exceeds the 10% level and even some points outside 25%. Such results could be expected as the crushing strength values are too variable. On the contrary, batch two meets totally the criteria of the USP.

Based on the following data presented in Fig. 5, the similarity factor f_2 was calculated.

For batch two, a comparison test is not required as the tablet releases almost 80% of the active compound artemether within 15 min.

For batch 1, the similarity factor f_2 was calculated, in which the points from 5 to 45 min were considered. A value of 34.10 was obtained, confirming the experimentally observed batch variation. The results of one batch are presented in Fig. 6.

A yellow colour in the samples was observed due to the extraction of lumefantrine, possibly partial, into the organic phase. Additionally, the dosage of artemether in the Coartem® tablet is lower that for the Artenam® tablets. Preconcentration steps are thus foreseen (see Section 2.4).

HPLC analysis could be performed as usual as the lumefantrine peak does not disturb the analysis of artemether.

In contrast with the dissolution profile of the tablets in water (maximal 15 mg (75%) release after 4 h), a total release of artemether was noticed in an acceptable time span. Within 80 min, 80% of artemether was released from the Coartem® tablet and a total 100% release occurred within 150 min. This method is therefore much faster then the one actually used by Novartis, by which 60% of the active is released after 3 h (internal communication).

The results suggest that the presented method is also useful and even more suitable for dissolution studies of Coartem® tablets.

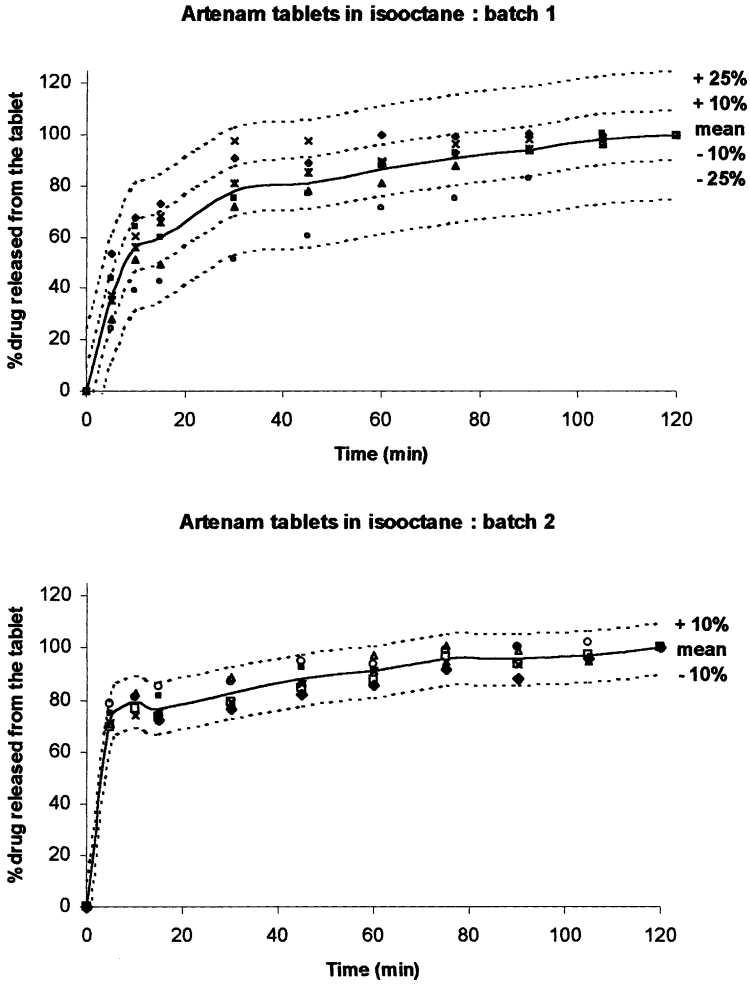

Fig. 5. Dissolution profile of two different batches of Artenam® tablets with the 'two phase partition-dissolution' method, using isooctane as extraction medium.

Finally, the 'two-phase partition-dissolution' method seemed to be suitable for the determination of dissolution profiles of artemether in tablets in general.

3.7.3. Optimization and validation of the conditions for the 'two-phase partition-dissolution' procedure for dihydroartemisinin tablets

3.7.3.1. Selection and determination of the volume of the organic phase for the 'two phase partition-dissolution' procedure for dihydroartemisinin tablets. Based on the solubility data of dihydroartemisinin in different organic solvents, the choice is rather limited. Only with chlorobutane, the sink conditions can be maintained in the two-phase partition-dissolution test and the volume of the organic phase was set on 150 ml instead of 100 ml. Glass vessels are preferred as plastic ones dissolve in chlorobutane. The more, the solvent disturbs the peak of dihydroartemisinin in HPLC analysis. Therefore, all samples were evaporated, and reconstituted in methanol.

3.7.3.2. Extraction of dihydroartemisinin. Before starting dissolution tests with the selected solvent, an extraction test is performed in the same ratio of the aqueous and organic phase as used in the dissolution test, namely 1:1 (v/v). The results from this test reveal

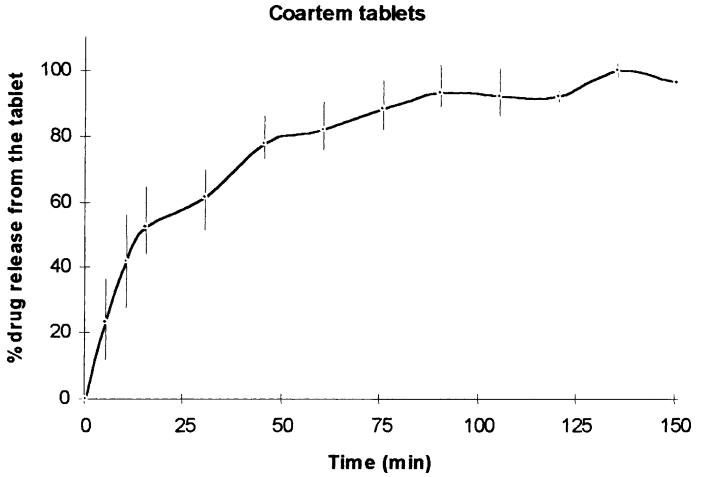

Fig. 6. Dissolution profile of artemether from Coartem® tablets, performed with the 'two-phase partition-dissolution' method (six samples).

an incomplete extraction from the aqueous phase into the organic phase, namely 87.44% (CV = 4.79%; ($n = 3$)), which should be considered in the calculation of the dissolution results.

3.7.3.3. Determination of the rotation speed of the paddle in the 'two-phase partition-dissolution' procedure for dihydroartemisinin tablets. In the preliminary tests with 'medium crushing strength' tablets, a turbulence was observed at the frontline between both phases. Sodium laurylsulfate 0.1% which wets the tablet, was added to the dissolution medium to avoid floating of tablets parts at the frontline. Moreover, the release of dihydroartemisinin is quite fast at 100 rpm (90% within 10 min), possibly too fast to discriminate different tablet hatches. (Fig. 7). Therefore, the rotation speed should be adapted to 50 rpm. Under these conditions, the tablets show a release of 60% within 15 min.

3.7.3.4. Determination of the drug release from 'low-medium-high crushing strength' tablets containing dihydroartemisinin. When comparing the release rate at 15 min, 78, 64 and 30% release were

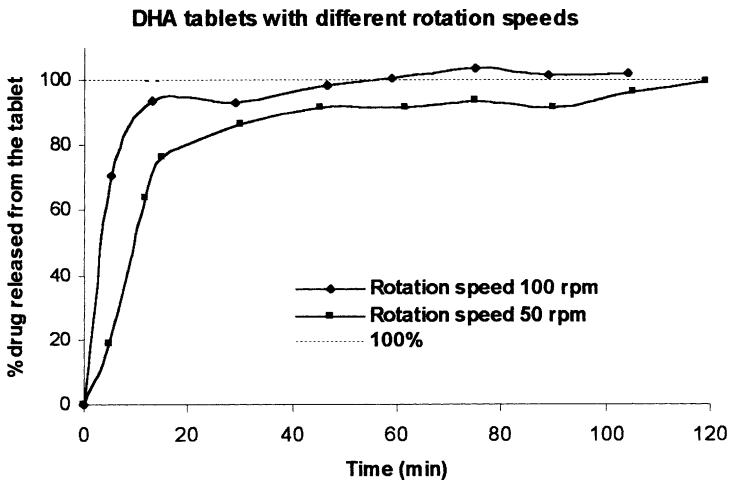

Fig. 7. Dissolution profile of DHA tablets with medium crushing strength at different rotation speeds with the 'two-phase partition-dissolution' method, using chlorobutane as extraction medium.

Table 4
Dissolution data of the DHA tablet batches with different crushing strength (CS), considered for determination of f_2

	Medium CS tablets (50 N)		Low CS tablets (100 N)		High CS tablets (150 N)	
	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)
0	–	–	–	–	–	–
5	56.35	8.35	18.26	52.06	5.66	2.95
10	81.63	13.92	42.17	56.51	10.69	44.30
15	78.08	16.69	63.71	34.93	29.95	16.93
30	85.93	11.72	85.58	7.46	51.94	23.34
45	85.41	1.56	89.50	3.00	58.69	9.23
60	100.61	6.64	92.06	3.98	63.59	22.04
75	–	–	93.02	4.54	76.25	3.33
90	–	–	95.89	3.37	75.91	6.03
105	–	–	95.74	2.65	86.48	5.34
120	103.61	3.54	98.07	2.66	2.29	4.33

f_2: low vs. medium: 26.37; low vs. high: 13.52; medium vs. high: 26.66

respectively noticed for the 'low, medium and high CS' tablets (Tables 3 and 4), allowing a clear distinction of the dissolution profile of different tablet hatches (Fig. 8). For the latter, the release seemed not to be finished within the period of investigation, namely 2 h. The spread of the results of the first points exceeds the requirements of the pharmacopoeia. Possibly, the preparation method (manual compression) contributed to that. Nevertheless, the factor f_2 results (see Table 6) confirm our observations strongly.

Based on the dissolution results, none of the DHA tablets fulfil the requirements of the EMEA, which states a 85% release within 15 min for an 'immediate release' tablet (EMEA, 2001).

3.7.3.5. Investigation of the suitability of the commercial tablet containing dihydroartemisinin, Cotecxin®, with immediate release characteristics. The dissolution profile of a commercial tablet Cotecxin®, investigated with the 'two-phase partition-dissolution' method, reveals that only 45% of dihydroartemisinin is released within 3 h. It is obvious that it does not fulfil the requirements of the EMEA (EMEA, 2001).

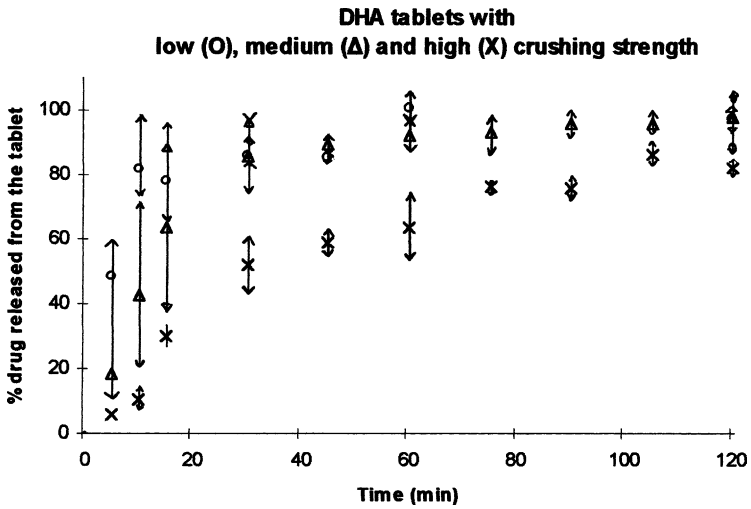

Fig. 8. Dissolution profiles of DHA tablets with different crushing strengths with the 'two-phase partition-dissolution' method, using chlorobutane as extraction medium.

4. Final conclusion

Several methods to evaluate the release profile of the very slightly soluble products, artemether and dihydroartemisinin, from a tablet, were investigated: the classic 'high volume' method, the flow-through method and the cosolvent method. As none of them can fulfil the Sink conditions throughout the test, they are not suitable.

Therefore, based on the work of Hoa and Kinget, a 'two-phase partition-dissolution' system was developed for both drugs, artemether and dihydroartemisinin; this method is characterized by the release of active compound in an aqueous phase, which is simultaneously extracted into an organic phase. The organic solvent part and its volume were selected as such that the organic solvent does not evaporate during the test, that the organic phase is in the upper part of the extraction system ($d_{\text{organic}}\text{solvent} < d_{\text{water}}$) that 'sink' conditions were assured in the organic phase; and that, if possible, a direct HPLC measurement could be performed at 215 nm (without an extra evaporation/reconstitution step in the procedure; cut-off near 200 nm). For artemether, the best results were obtained with iso-octane. Repeatability and discrimination ability tests in the selected solvent showed good results. The difference in release rate characteristics of the investigated batches of Artenam® tablets was observed and could be proven via the similarity factor f_2 (value <50). Similar tests were performed on dihydroartemisinin tablets. Only one organic solvent, with an enhanced volume compared to artemether (150 ml), chlorobutane, seemed to be suitable to perform the 'two-phase partition-dissolution' method on dihydroartemisinin. Two disadvantages should be noticed, compared to artemether, namely glass vessels should be used and a special sample treatment (preconcentration and reconstitution in methanol) is required for HPLC analysis.

Discrimination ability of the method for dihydroartemisinin, performed with self-made tablets of different crushing strength, was confirmed via the similarity factor f_2. Finally, both two-phase partition-dissolution methods for artemether and dihydroartemisinin seemed to be suitable to evaluate the dissolution profile from marketed tablets.

The commercial Cotecxin® tablets seemed not to be conform to the Eur. Pharm. requirements for immediate release tablets.

In contrast with the method of Hoa and Kinget, both methods allowed easy sample taking from the upper phase. This method seemed to be more suitable than the methods actually used in registration (water), for artemether, as it allows 100% release within an acceptable time span. Possible this method is a good base to develop a dissolution method for other non-water or slightly water soluble drugs.

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