

DETERMINATION OF DICLOFENAC RELEASED FROM SUPPOSITORIES USING UV SPECTROPHOTOMETRY, SPECTRA DERIVATIVE SPECTROPHOTOMETRY AND HPLC

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Abstract: Spectrophotometric method is often recommended in dissolution testing of not only tablets or capsules but also suppositories. Dissolution rate of diclofenac sodium was studied using a pharmacopoeial flow-through apparatus and pH 7.3 buffer. For two lipophilic suppository bases (Witepsol W-32 and H-19) dissolution rate was overestimated with the spectrophotometric zero-order method in comparison to HPLC method, although the difference was statistically significant only in the case of the Witepsol W-32 basis. On the other hand, first derivative spectrophotometry allowed for elimination of the interference of the suppository lipophilic components dissolved in the buffer medium and no difference was observed between the results of this analysis in comparison with HPLC results.

Keywords: dissolution test, suppository, diclofenac, spectrophotometry, HPLC

In vitro testing of drug release from pharmaceutical dosage forms is a common step of a routine control, as well as an initial phase of a product development process. In addition, dissolution test provides valuable information on physicochemical stability of the product. Although oral forms i.e. tablets and capsules are mainly tested for drug release on a routine base, the test is also recommended for some other products including suppositories. The *in vitro* release test for suppositories has always posed many problems, including choice of a suitable apparatus, test conditions and interpretation of the results (1). Even when the flow-through apparatus dedicated for this dosage form was introduced to European Pharmacopoeia, other methods like basket or beaker methods, with or without dialysis membrane, are still in use (2-4).

During the test samples of the dissolution medium are collected and analyzed by a suitable analytical method, what can be a difficult step as the release of active substance from suppositories is accompanied by dissolution of the components of a suppository basis. In suppositories with lipophilic bases, besides lipids, also surfactants and antioxidants, as well as colorants may be present and they can dissolve in the acceptor fluid during the test. Their release rate is unknown and their amount in the analyzed samples varies in an unpredicted man-

ner. Thus it is important to choose for the analysis of the active substance a method where these components do not interfere with the measurements.

UV spectrophotometry is in wide use in the quantitative analysis of the active substances in dissolution tests. Determination of diclofenac by UV-VIS spectrophotometry is recommended for example in a pharmacopoeial monograph of diclofenac sodium delayed-release tablets (USP 28). However, this method can be badly affected by significant interferences caused by the excipients accompanying the active substance. Spectral overlap and non specific irrelevant absorption affect the interpretation of data leading to variable intercepts on the absorbance axis and systematic errors in the graphs of absorbance versus concentration (8). Suitability of the method has to be confirmed in a validation procedure, however, it can be difficult due to the above mentioned unpredictable dissolution rate of suppository basis. In our studies, suitability of UV spectrophotometry for dissolution test of diclofenac sodium from lipophilic suppositories was evaluated by comparison with the results obtained using HPLC analysis.

In comparison with conventional spectrophotometric determinations, derivative spectrophotometry has proved to be of a great value in eliminating the interference from excipients and co-formulated

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drugs. The derivate technique presents significant advantages over the conventional absorption method (9). The method was proposed for quantitative analysis of many drugs, among them for the assay of diclofenac in pharmaceutical forms (5-8), but to our knowledge comparison of this method with the conventional spectrophotometry to study release profiles of diclofenac or other active substances from suppositories was not reported so far.

EXPERIMENTAL

Materials and chemicals

Two types of suppositories were used in the study: commercial product Diclac 100 (Hexal, Holzkirchen, Germany) and compounded suppositories, both containing 100 mg of diclofenac sodium. Diclac suppositories are manufactured on a lipophilic basis Witepsol W-32 (melting point 32.0-33.5°C), they are 1 g in weight and the manufacturer does not reveal any other excipients. Compounded suppositories were prepared using diclofenac sodium (Amoli Organics, Mumbai, India) and the lipid – Witepsol H-19 (a gift from Sasol, Hamburg, Germany) with melting point 33.5-35.5°C. The suppositories with a weight of 2 g were prepared by a moulding method.

Buffer solution pH 7.3 used as a dissolution medium was prepared by dissolving 6.8 g of potassium dihydrogen phosphate and 1.52 g of sodium hydroxide (POCh, Gliwice, Poland) in 1000 mL of water. For the HPLC analysis buffer solution pH 2.5 was prepared by dissolving 0.8 g of potassium dihydrogen phosphate in 1000 mL of water and adjusting pH with phosphoric acid (POCh, Gliwice, Poland). Methanol was HPLC grade (POCh Gliwice, Poland).

Apparatus and conditions

The Ph. Eur. flow-through apparatus for testing suppositories (PharmaTest, Hainburg, Germany) was used in a dissolution experiment. The system was placed in a thermostated bath and the acceptor fluid was pumped through the dissolution chambers using a peristaltic pump (MPC, Ismatec, Wertheim-Mondfeld, Germany).

A spectrophotometer UV-VIS Jasco V-530 (Jasco, Tokyo, Japan) was employed for all spectral measurements. Absorption spectra were recorded at a scan speed of 200 nm/min between 230-330 nm. The compensation liquid was phosphate buffer pH 7.3. Analytical wavelengths were 275 nm and 261 nm for conventional (zero-order) and first derivative methods, respectively.

The HPLC system (Merck Hitachi, Darmstadt, Germany) consisted of an integrator D-2500A, detector UV-VIS L-4250, pump L-6200A and column Lichrospher 100 RP 18 (5 μ m, 25 \times 4 cm; Merck). The analysis was performed using as a mobile phase mixture of methanol and phosphate buffer pH 2.5 (76:24, v/v). The mobile phase flow rate was 1.0 mL/min. Detection was performed at 254 nm.

In vitro release study

The test was performed according to the general recommendations given in Ph. Eur. 5. A single suppository was placed in the chamber of the flow-through apparatus. The following experimental conditions were set up: phosphate buffer pH 7.3 was used as a dissolution medium, with flow rate of 100 mL/h (\pm 1.7 mL/h) and temperature was maintained at 37.0 \pm 0.5°C. Fractions of the dissolution medium were collected in 15 min or 30 min intervals up to 120 min. For the Witepsol W-32 (Diclac) suppositories the dissolution fluid was also collected after 240 min. The experiment was repeated 6 times for each type of the suppositories.

Analysis of diclofenac sodium in the dissolution medium

For calibration curves diclofenac sodium standard solution was diluted with phosphate buffer pH 7.3 or methanol for spectrophotometric and HPLC methods, respectively, to obtain concentrations in the range 5-25 μ g/mL.

The assay of diclofenac sodium in the collected fractions of the dissolution medium was performed both spectrophotometrically and by HPLC within 30 h. It was confirmed that the results were reproducible within this time. The concentrations required for the spectrophotometric or HPLC analysis were obtained by suitable dilutions of the samples with phosphate buffer pH 7.3 or methanol, respectively.

Statistical analysis

The statistical significance of differences between methods was assessed by Student's t-test with $p < 0.05$ being considered as statistically significant.

RESULTS AND DISCUSSION

Diclofenac UV spectra in phosphate buffer are presented in Figure 1. The calibration curves were constructed at the wavelengths corresponding to maxima at analytical wavelengths 275 nm and 261 nm for zero-order and first-order derivative spectra,

respectively. The linearity of the method was demonstrated (correlation coefficients, R^2 are close to 1.0 as presented below) for standard solutions containing diclofenac sodium in the concentration range 5-25 $\mu\text{g/mL}$. Quantification of diclofenac sodium was based on the calibration curves representing a dependence of the absorbance (y) on concentration (x , $\mu\text{g/mL}$):

$$\text{zero-order spectrum: } y = 0.0334x - 0.0057 \quad (R^2=0.9994)$$

$$\text{first derivative spectrum: } y = 0.00096x - 0.00005 \quad (R^2=0.9992)$$

Dissolution test was performed for two types of suppositories containing the same dose of the active substance, i.e. 100 mg, but prepared on different types of the lipophilic bases and with different total weights: commercial 1 g Diclac suppositories were manufactured on Witepsol W-32 whereas Witepsol H-19 was used for the compounded 2 g suppositories. Witepsol is a brand name for a mixture of mono- di- and triglycerides, commonly used as suppository bases. However, Witepsols differ in the ratios of different types of glycerides, what results, for example, in different melting points as indicated in the experimental section.

The effect of lipids used as suppository bases on the recorded spectra was not measured directly since their solubility in the phosphate buffer is unpredictable and depending on many factors like, for example, melting rate or hydrodynamic conditions during the dissolution test. The effect of the dissolved suppository lipid components can be seen

indirectly by comparison of the results of diclofenac analysis in the dissolution medium performed with different methods. The HPLC method, based on separation principle, was used as a reference method for spectrophotometry.

The HPLC method employed the experimentally determined calibration curve presented below and linearity of the method was demonstrated in the concentration range 5-25 $\mu\text{g/mL}$.

$$\text{HPLC: } y = 10.942x + 10.159 \quad (R^2=0.9992)$$

where: y – is an area (in thousands) of diclofenac peak (retention time, t_R about 7 min).

Table 1 presents the amounts of diclofenac sodium determined in the collected fractions of the dissolution fluid using the three methods and expressed as a per cent of the total dose of the drug in suppository. The average values with standard deviations (SD) are given. The results obtained with the spectrophotometric methods are compared with the amounts determined with the reference i.e. HPLC method and the difference is shown as an error (E) and as a relative error RE. The E and RE values were calculated using the following equations:

$$E = Q_{\text{spectr}} - Q_{\text{HPLC}}$$

$$RE = \frac{Q_{\text{spectr}} - Q_{\text{HPLC}}}{Q_{\text{HPLC}}} \times 100\%$$

Where: Q_{spectr} – per cent of diclofenac released, measured spectrophotometrically,

Q_{HPLC} – per cent of diclofenac released, measured by HPLC method.

Table 1. The amounts of diclofenac sodium (% of the total dose) released in time intervals from suppositories prepared with Witepsol H-19 and Witepsol W-32 bases (n = 6) measured by HPLC, conventional, and first derivative spectrophotometry.

Time [min]	HPLC		Spectrophotometry				Derivative spectrophotometry			
	Mean	SD	Mean	SD	E	RE	Mean	SD	E	RE
Witepsol H-19										
15	9.39	3.61	9.67	3.78	0.27	2.93	9.26	3.67	-0.13	-1.36
30	27.33	2.75	28.17	2.54	0.84	3.07	27.53	2.54	0.20	0.75
45	16.78	1.64	17.57	1.93	0.78	4.67	17.11	1.85	0.33	1.94
60	7.93	0.98	8.37	1.15	0.44	5.54	8.16	1.09	0.23	2.93
90	7.16	0.74	7.87	1.21	0.71	9.92	7.68	1.31	0.52	7.28
120	3.22	0.45	3.43	0.48	0.21	6.58	3.35	0.42	0.13	4.03
Witepsol W-32										
15	37.27	3.99	41.20	3.93	3.93	10.55	35.86	3.69	-1.41	-3.77
30	27.65	1.94	28.70	2.28	1.05	3.80	26.50	2.16	-1.16	-4.18
60	13.08	1.93	14.81	2.28	1.73	13.26	13.33	2.38	0.26	1.95
90	4.48	1.70	4.67	1.79	0.19	4.22	4.29	1.88	-0.19	-4.27
120	2.34	1.34	2.52	1.36	0.18	7.84	2.37	1.36	0.03	1.43
240	6.14	2.83	6.26	3.27	0.12	1.97	6.04	3.13	-0.10	-1.61

SD – standard deviation; E – difference between the spectrophotometric and HPLC result; RE – relative error (see text)

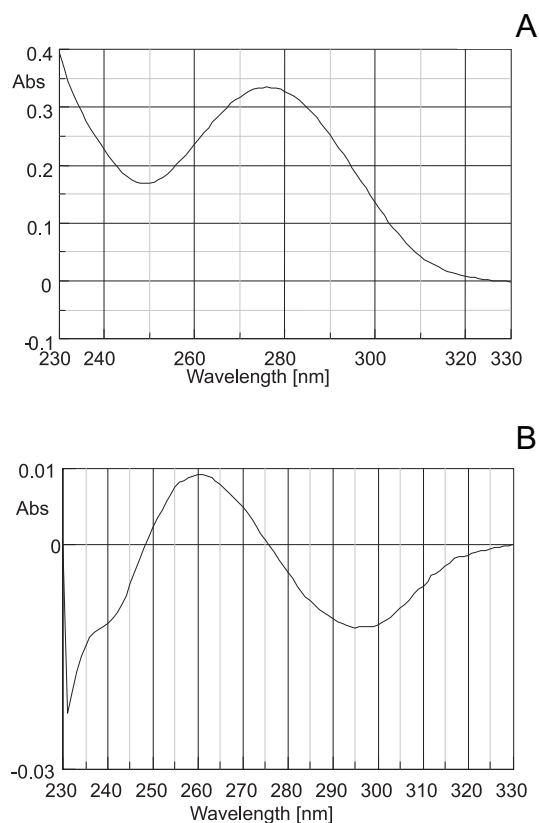


Figure 1. Absorption spectra of diclofenac sodium (10 µg/mL) in phosphate buffer pH 7.3: A – zero-order spectrophotometry, B – first-order derivative spectrophotometry.

For both types of suppositories first derivative spectrophotometry gave results closer to those obtained with HPLC. In the case of Witepsol W-32 suppositories (Diclac) the difference between derivative spectrophotometry and HPLC was not larger than 1.41% of the drug released (after 15 min) and when the difference is expressed by a relative error (RE) the maximum deviation is 4.27% (in the fluid collected after 90 min). Regarding the conventional spectrophotometric measurements the maximum difference (E) is also observed for the portion of the fluid collected after 15 min (3.93%), while RE was the largest after 60 min (13.26% of the dose measured by HPLC). For Witepsol H-19 suppositories the differences were smaller but still the values obtained with the conventional spectrophotometry were higher than those measured with first derivative method: the average RE values for first derivative spectrophotometry was 3.0% and for conventional spectrophotometry – 5.4%. For both types of the suppository bases all values obtained with zero-order UV method exceeded those measured by HPLC, while this trend was not a case for the deriv-

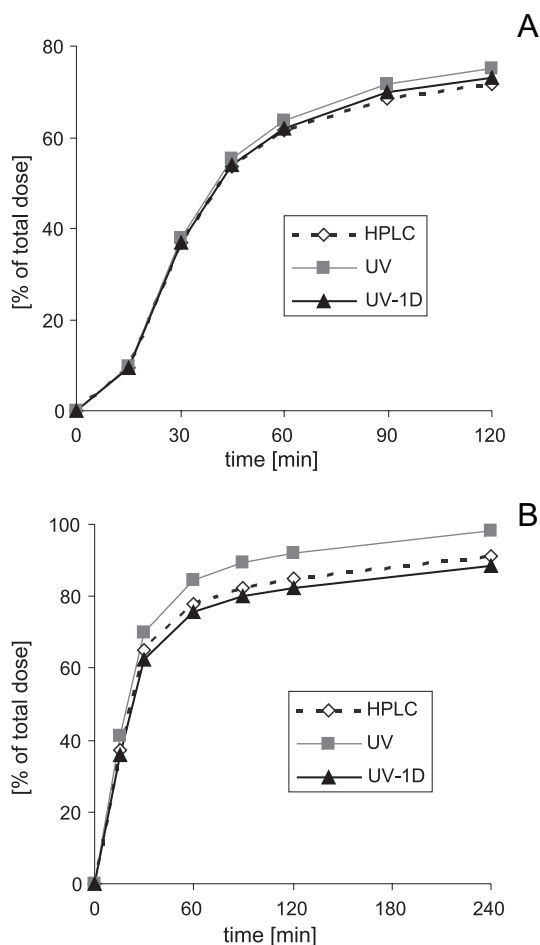


Figure 2. Dissolution profiles of diclofenac sodium from Witepsol H-19 (A) and Witepsol W-32 (B) suppositories determined by using three methods: HPLC, UV spectrophotometry and first derivative spectrophotometry (UV-1D).

ative spectrophotometry values, especially when W-32 basis was considered. However, in spite of these observations none of the difference was statistically significant when the values were compared using Student's t-test.

Table 1 shows the amounts of diclofenac sodium released within the time intervals, but for illustrating the release profiles these values are summarized and the cumulated amount released is used for demonstrating dissolution test results. The obtained cumulative dissolution curves are shown in Figure 2. The release of diclofenac from Witepsol W-32 basis was faster than from Witepsol H-19 basis, with more than 60% of the drug released within 30 min, while in the latter formulation the same amount was released only after 60 min.

For Witepsol W-32 formulation the cumulative amount of the released drug determined with the conventional UV spectrophotometry is higher,

whereas both curves obtained with derivative UV spectroscopy and HPLC are overlapping. With the statistical test it was demonstrated that the difference for HPLC and conventional UV spectrophotometry was significant in the time range between 15 and 90 min. For the Witepsol H-19 formulation the difference was not significant. Thus, even insignificant differences in the measured amounts of the active substance released in time intervals (Table 1) may lead to significant differences in the cumulated profiles as occurred for the Witepsol W-32 suppository.

It may be concluded that Witepsol W-32 interferes with the analytical method by conventional spectrophotometry to a larger extent than Witepsol H-19. Larger difference between the results of UV and HPLC measurements for W-32 (Diclac) suppository than for Witepsol H-19 formulation, despite of the smaller mass of the units, indicates that more components of the lipophilic basis are dissolved from Witepsol W-32 suppository. The difference can be explained with the chemical characteristics of Witepsol W-32 and H-19. The former has lower melting point and higher hydroxyl value, what can be correlated with higher content of the fraction soluble in buffer pH 7.3 (free fatty acids and mono- and diglycerides).

However, irrespective of the solubility of some components in water, the results calculated using the first derivative were reliable. The derivate technique presents significant advantages over the conventional spectrophotometry. This method, simple and

rapid, is recommended when dissolution is tested using spectrophotometry, especially when suppository bases demonstrate slight solubility in water.

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