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Flow injection extraction applied to dissolution rate studies of felodipine tablets

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Abstract: A flow injection analysis (FIA) extraction method has been developed for the analysis of felodipine tablets in connection with dissolution rate testing. The water-soluble oxidation product of felodipine, a pyridine derivative, was extracted into chloroform and measured at 275 nm spectrophotometrically. The FIA-extraction method has been compared with the present liquid chromatographic (LC) method. The sampling rate for the FIA-extraction (60 samples h^{-1}) is 5 times higher than for the LC method. The FIA-extraction method has a standard deviation of 1% for both standards and samples which is the same as for the LC method.

Keywords: *Flow injection analysis (FIA); extraction; dissolution testing; felodipine; calcium antagonist.*

Introduction

The flow injection analysis (FIA) technique is well suited for pharmaceutical quality control due to its capacity for handling a large number of samples [1, 2] such as are often generated in the determination of dissolution rates. Such a test is now required for most solid dosage forms. Koupparis *et al.* have shown the applicability of FIA in dissolution studies [3–7]. One of the characteristics of flow injection is that the sample can undergo some reaction or treatment on its way from the injector to the detector. If the reaction is non-selective, or if interferences appear, a separation step could be helpful. Samples with an interfering matrix can, for example, be treated by liquid–liquid extraction in order to improve the possibilities for proper detection. FIA-extraction was first described by Karlberg and Thelander [8]. The aqueous stream is segmented by an organic phase so that analyte transfer can take place. After phase separation the organic phase is led through the detector. This technique has now developed into an established sample conversion procedure [9–12]. In this study the FIA-extraction approach is considered for the dissolution rate determination of felodipine tablets (a calcium antagonist for the treatment of hypertension). The method is also compared with a liquid chromatographic (LC) method which was developed previously.

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Experimental

Dissolution method

The dissolution of felodipine tablets was tested in 0.1 M sulphuric acid containing 5 mM of cerium sulphate using a USP dissolution Apparatus 2 with a rotational speed of 50 rpm, a temperature of 37°C and a test volume of 500 ml. Samples of 10 ml vol were withdrawn and filtered using a 0.8 μm filter (Nuclepore) after 10, 20, 30 and 60 min.

Reagents

Tablets containing 5 and 10 mg of felodipine (3,5-pyridinedicarboxylic acid,4-(2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethyl-, ethyl methyl ester) were produced at AB Hässle. The pyridine derivative reference substance (3,5-pyridinedicarboxylic acid, 4-(2,3-dichlorophenyl)-2,6-dimethyl-, ethyl methyl ester) was synthesized at AB Hässle. All other chemicals were of analytical grade.

Flow injection system

A general flow diagram of the FIA-extraction system is shown in Fig. 1. Two LC pumps (Beckman 110A, Berkeley, USA) were used to pump water. Each pump was equipped with an LC column in order to give sufficient working pressure for the pulsedampers. The aqueous carrier stream was deionized water and the stream of organic phase (chloroform) was produced by pumping deionized water into a displacement bottle (DB) (Tecator, Sweden). The flow rate for the aqueous stream was 2.0 ml min^{-1} and for the organic stream 1.0 ml min^{-1}. The sample solution was injected into the water stream using a Rheodyne 7410 injector(I) equipped with a 2- or 5-μl sample loop. The tubing and connectors up to the segmentor were made of stainless steel. The extraction occurred in the extraction coil (E) which was made of 2 m 0.8 mm, i.d. PTFE tubing. The segmented stream was separated in a phase separator (S) exploiting a PTFE 0.2 μm membrane (Millipore, FGLP) [13]. The volume of the cavity for the ingoing segmented stream in the separator was 42 μl and the volume for the outflowing organic phase was 8 μl. A fraction (about 70%) of the organic phase was led through a spectrophotometric detector (D) (Lambda-Max mod. 481, Waters). The analyte was detected by UV absorbance at 275 nm. The peak heights were evaluated using a chromatographic data system (Nelson Analytical Inc.).

LC system

The dissolution of felodipine tablets was also followed with a reversed-phase LC system. The mobile phase consisted of methanol (50 vol%) and 0.2 M perchloric acid (50 vol%). The sample (20 μl) was injected by means of an autoinjector (WISP mod. 701, Waters) and separation took place in a RP-8 (5 μm) guard column (Brownlee Labs) with a length of 30 mm and 2.1 mm, i.d. The same type of detector as described above was used. The peak area was evaluated by an integrator (SP4100, Spectra-Physics).

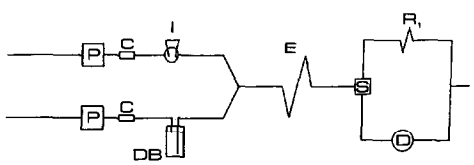

Figure 1
The FIA-extraction manifold. P, LC pumps; C, LC columns; I, injector; DB, displacement bottle; E, extraction coil; S, phase separator; R_1, restrictor; D, detector.

Results and Discussion

Batch extraction

In order to follow the dissolution of the felodipine tablets, felodipine must be chemically modified into a water-soluble substance to maintain sink conditions [14]. This is done by oxidation with $Ce(IV)(SO_4)_2/H_2SO_4$ solution into a pyridine derivative as shown in Fig. 2. The pyridine derivative is charged and soluble in acidic solution.

Spectra of a felodipine tablet (A) dissolved in $Ce(IV)(SO_4)_2/H_2SO_4$ solution, and the pyridine derivative (B) dissolved in 0.1 M H_2SO_4 are shown in Fig. 3. Ceric ions absorb strongly in the UV-range and it is impossible to make a differential spectrophotometric measurement to determine the pyridine derivative. Spectra of the solution from the dissolved felodipine tablet batch extracted with chloroform (A) and the pyridine derivative dissolved in chloroform (B) are shown in Fig. 4. It is evident that extraction is

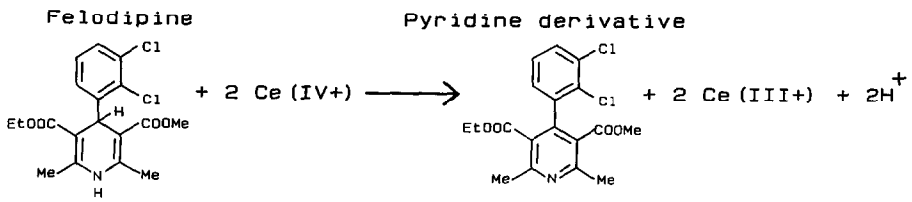

Figure 2
Oxidation of felodipine with cerium to the soluble pyridine derivative.

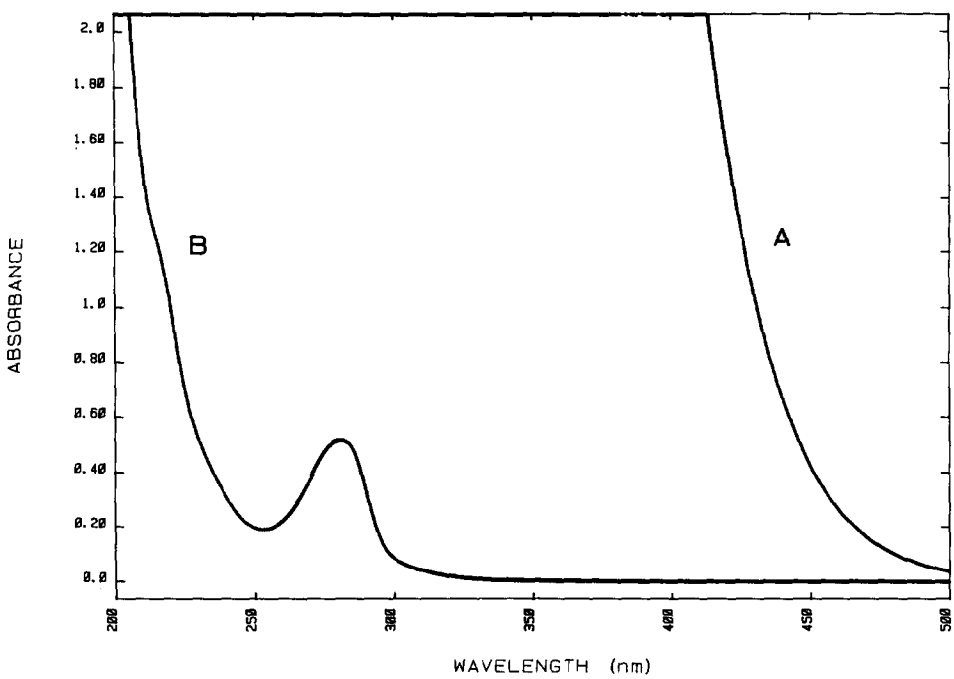

Figure 3
Spectra of: (A) one 10-mg felodipine tablet in 500 ml 0.1 M H_2SO_4–0.005 M $Ce(SO_4)_2$; filtered through 0.45 μm; (B) 0.02 mg ml^{-1} pyridine derivative in 0.1 M H_2SO_4.

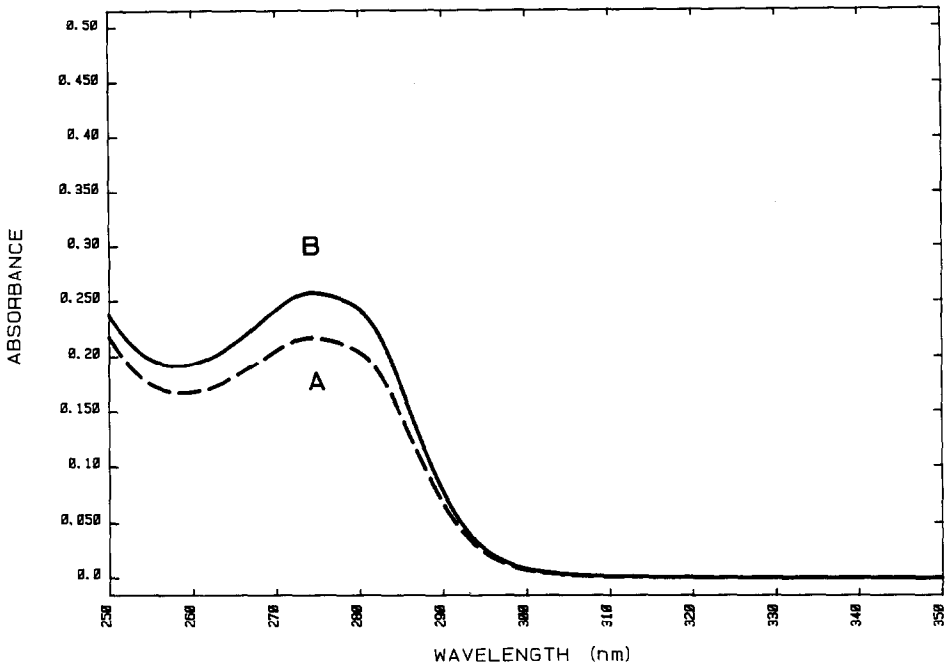

Figure 4
Spectra of: (A) chloroform extract of a felodipine tablet dissolved in 0.1 M H_2SO_4–0.005 M $Ce(SO_4)_2$, filtered through 0.45 μm; (B) pyridine derivative dissolved in chloroform.

a useful method for the separation of the pyridine derivative from the ceric sulphate solution of the dissolution baths and that the pyridine derivative is hydrophobic enough to be extracted from an acidic solution.

Flow injection extraction

The influence of the extraction-coil length on the FIA-extraction of the pyridine derivative from an acidic solution (0.1 M H_2SO_4) into chloroform is illustrated in Fig. 5. At a total flow rate of 3 ml min^{-1} it takes 1 m of 0.8 mm, i.d. PTFE tubing, corresponding to 10 s residence time, to reach complete extraction. Increasing the length of the tubing over 1 m does not affect the peak height. Evidently, the sample dispersion in the extraction coil is small when using chloroform as the organic phase. Thus flow rate and extraction-coil length can be varied within relatively wide limits, maintaining small dispersion.

The possibility of concentrating the pyridine derivative into the organic phase by FIA-extraction has been tested (Fig. 6). The flow rate of chloroform and the sample volume have been held constant while the aqueous carrier flow rate has been varied. The relation between peak height and aqueous flow rate is almost linear. Thus the distribution ratio is high enough to give a high yield even at high aqueous/organic flow ratios. The possibility of concentrating the analyte into the organic phase is important as it permits a decrease in the sample volume whilst maintaining sensitivity.

The fraction of the organic phase reaching the detector is an important variable in FIA-extraction. It determines the dispersion in the phase separation and detection part

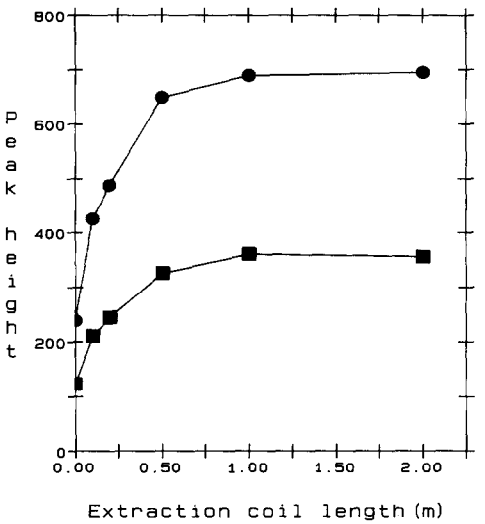

Figure 5
Influence of the extraction-coil length on the FIA-extraction of the pyridine derivative. Flow rates: chloroform, 1.0 ml min^{-1}; aqueous carrier, 2.0 ml min^{-1}. Segment length: 3 mm, organic phase. Extraction tubing: 0.8 mm i.d. PTFE. Sample: pyridine derivative, ■, 10 mg l^{-1}; ●, 20 mg l^{-1}.

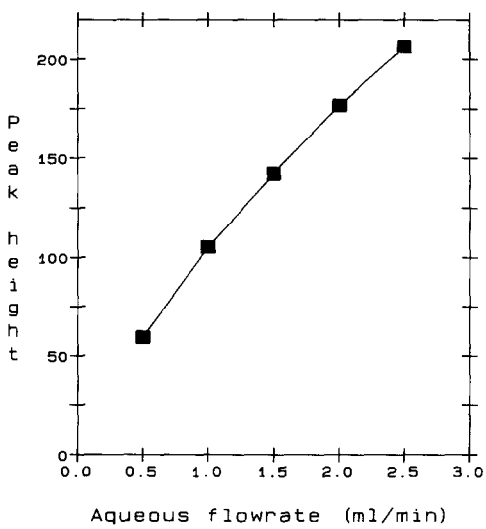

Figure 6
Influence of the aqueous/organic flow ratio on the peak height. Sample: pyridine derivative, 0.02 mg ml^{-1}. Organic phase chloroform, 1.0 ml min^{-1}.

of the flow system and consequently affects the sensitivity and sampling frequency of the system. To achieve phase separation a differential pressure must be applied over the membrane in the separator. This is done by placing a restrictor in the aqueous waste flow from the separator (R_1). The relation between the restrictor length (R_1, Fig. 1) and the phase separation efficiency is shown in Fig. 7. As can be expected from flow resistance considerations the relation is linear at a low separation efficiency but slightly curved when it reaches 100%.

The relation between peak height and separation efficiency is shown in Fig. 8. When the separation efficiency becomes <50% the peak height decreases rapidly. Between a separation of 60–90% the peak height changes <10%. When the loss of organic phase approaches zero the peak height increases sharply. The reason for this is that the inlet chamber of the separator stops contributing to the dispersion [15].

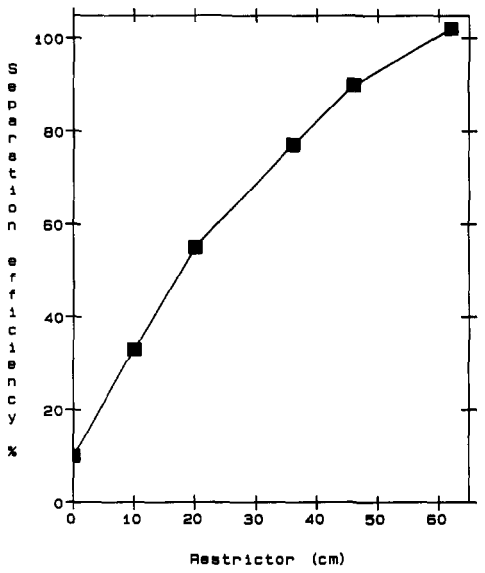

Figure 7
Influence of the restrictor R_1 on the phase separation efficiency. Restrictor: 0.35 mm i.d. PTFE. Flow rates: aqueous carrier, 2.0 ml min^{-1}; chloroform, 1.0 ml min^{-1}.

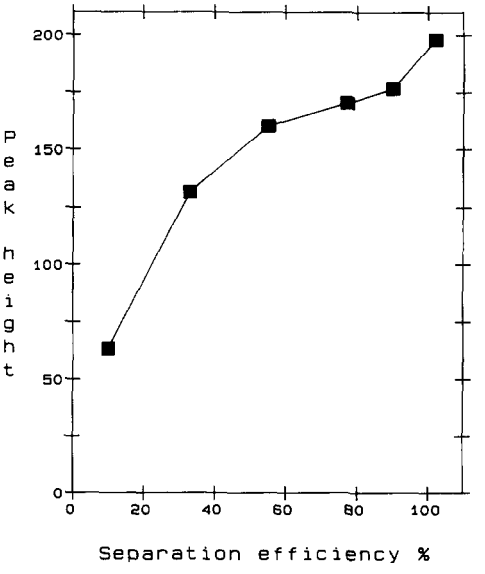

Figure 8
Relation between phase separation efficiency and peak height. Flows as in Fig. 7. Sample: pyridine derivative, 20 mg l^{-1}.

The formulation of the felodipine tablets contains several inactive ingredients. The lifetime of the separator membrane may be affected if large quantities of these substances are introduced into the flow system. These can be adsorbed onto the membrane and gradually destroy its phase separating ability. Therefore, the sample volume should be kept as small as possible. In order to get sufficient sensitivity the aqueous/organic flow ratio should be high and the dispersion in the phase separation/detection system should be low. The choice of an aqueous/organic flow ratio of 2:1 gave a sensitivity increase of a factor 1.8. At this flow rate and flow ratio it was possible to produce small and even segments with an ordinary T-piece segmentor. The flow rate of

the organic phase could be decreased below 1 ml min^{-1} in order to increase the concentration factor. This would, however, make the peaks broader (in time units) and consequently decrease the sampling frequency. Choosing a separation efficiency between 60–90% gave almost maximum sensitivity.

In addition, small fluctuations in the separation efficiency will have the smallest influence on the precision of the measurement. On these grounds, the extraction system variables were set as follows: extraction coil, 2 m (0.8 mm, i.d.); aqueous flow, 2.0 ml min^{-1}; organic flow, 1.0 ml min^{-1}; separator outflow restrictor, 0.48 m (0.35 mm, i.d.).

The ratio between surface area and liquid volume is high in FIA compared with batch methods. In an extraction from aqueous to organic phase, the analyte must either be lipophilic or be made so by formation of a complex or an ion-pair. In the parts of the FIA manifold where no organic phase is present the analyte can be adsorbed onto the tubing walls if the tubing is of a lipophilic material such as PTFE. For the pyridine derivative, this was verified by first injecting standard in 0.1 M H_2SO_4 with a PTFE injection loop and then only 0.1 M H_2SO_4. This showed that the second injection gave a small peak. Some of the analyte was obviously adsorbed in the injection loop and desorbed again at the second injection. This was avoided by replacing the PTFE tubing with steel tubing in the injector, injection loop and the tubing up to the segmentor.

Phase separation

The key to successful FIA-extraction is a properly working phase separation process. When measurement is performed in the organic phase, there are two requirements for the phase separation: firstly, the separator must deliver a stream of the organic phase free from droplets of aqueous phase that can disturb the detection. Secondly, the separation efficiency must be acceptably high for sensitivity reasons as shown in Fig. 8.

The ability of the phase separator membrane to exclude water can be expressed by the "water intrusion pressure". This depends on: (1) the pore size of the membrane, for a dry membrane it is 2.8 bar at 0.2 μm and 0.7 bar at 1.0 μm (manufacturers specification, Nucleopore Corp.); (2) the hydrophobicity of the organic phase, the higher the hydrophobicity the higher the pressure; and (3) the presence of substances which can adsorb on the membrane and decrease the water intrusion pressure.

A typical run at an early stage of the method development (the pumps, injector, displacement bottle, separator and detector are the same as in the final manifold) is given in Fig. 9A. As can be seen, the baseline was slightly unstable and for every turn of the injector, a small peak occurred. When the injector was turned from "load" to "inject", and vice versa, the aqueous carrier stream was blocked for a short period of time. During this period the pressure in the carrier stream built up quickly as the pump continued to force liquid into the blocked system. When the injector came to its new position, the pressure was released and the pressure pulse travelled through the flow system. On the aqueous side, the system consists of steel tubing and high pressure components with very small flexibility. Thus the pressure will build up more quickly during the injector actuation than for a conventional FIA system with a peristaltic pump and flexible tubing. From the injector onward, the system consisted of wide-bore tubing with low flow resistance. This meant that the pressure pulses could travel relatively undisturbed from the injector to the phase separator.

Figure 7 shows that there is an almost linear relationship between the differential pressure over the membrane created by the restrictor (R_1) and the separation efficiency. The separator membrane is thus exposed to a relatively high constant pressure. The

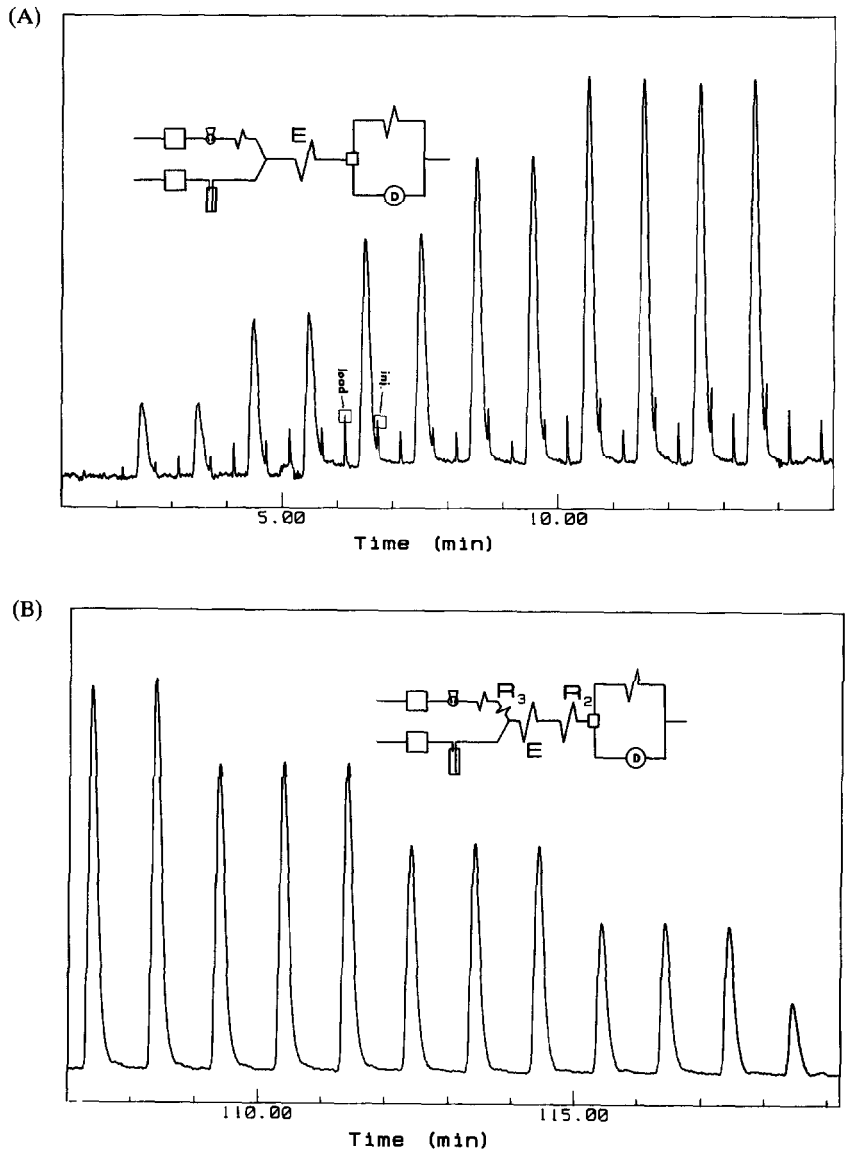

Figure 9
(A) Effect of pressure pulses at injection without restrictors, the extraction coil is 2 m 0.8 mm i.d. PTFE. (B) Effect of pressure pulses at injection with restrictors; R_2 is 1 m 0.35 mm i.d. PTFE, R_3 is 0.2 m 0.1 mm i.d. stainless steel.

additional pressure of a passing pressure pulse can be high enough to force some water through the membrane causing small peaks as in Fig. 9A.

To decrease the pressure pulses restrictors were introduced prior to the segmentor and the separator. The increased flow resistance forced the slightly flexible PTFE tubing in the extraction coil to take up most of the pressure pulse. This results in the peaks due to actuation of the injector disappearing completely, as can be seen in Fig. 9B.

Validation of the FIA-extraction method

The FIA-extraction at the beginning of a dissolution test of six individual tablets containing 5 mg of felodipine each, is shown in Fig. 10. The run started with standards corresponding to 150,100 and 50% dissolved felodipine and continued with 10-min samples, a 50% standard and 20-min samples for two tablets.

The calibration graph was linear and the peaks well separated at a sampling rate of 60 injections h^{-1}. In order to evaluate the accuracy of the FIA-extraction it was run in parallel with the present LC method. Thus Table 1 shows the complete FIA and LC results for the tablets in Fig. 10. The highest absolute difference was 6%-units and the highest relative difference was 21%, occurring only at the 10-min results.

A comparison of the FIA-extraction and LC analysis results for 30 felodipine tablets, each measured after 10, 20, 30 and 60 min in the dissolution bath, is shown in Fig. 11.

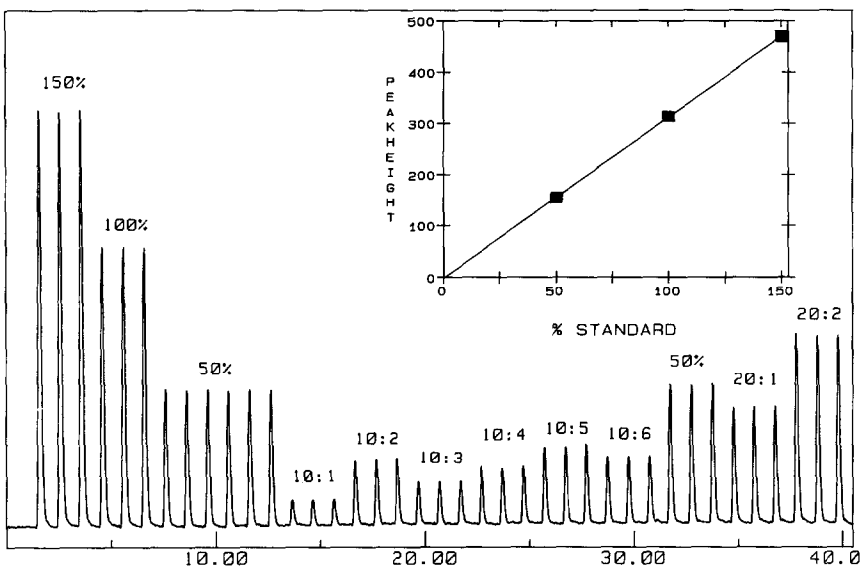

Figure 10
FIA-extraction run from the beginning of a dissolution test of six 5-mg tablets. Sample volume, 5 μl.

Table 1
Comparison of FIA-extraction and LC for a dissolution test of six 5-mg felodipine tablets. FIA injection volume 5 μl

Time:	10 min			20 min			30 min			60 min		
	FIA	LC	%	FIA	LC	%	FIA	LC	%	FIA	LC	%
Tablet No.												
1	11	9	21	42	39	8	77	73	6	103	99	4
2	25	22	12	67	63	5	89	83	6	103	100	3
3	17	14	14	53	50	5	80	78	3	102	98	4
4	22	19	13	65	62	6	85	82	4	103	98	4
5	29	27	6	70	67	5	86	85	2	101	97	4
6	25	23	9	67	64	5	87	83	4	101	97	4
Mean:	21	19	12	61	57	6	84	81	4	102	98	4

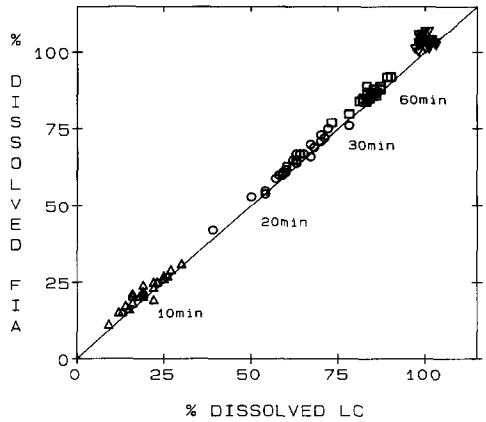

Figure 11
Comparison of data from dissolution tests of 30 tablets analysed by FIA-extraction and LC.

The least-squares fitted straight line for the 120 data points was $y = 1.0097*x + 1.44$, where y is % dissolved felodipine measured with FIA-extraction and x measured with LC. The FIA-extraction results lie 1.4% over the LC results, which can be explained by a small interference from the placebo. As can be seen from the graph, this difference is far less than the difference in dissolved amounts between individual tablets.

The relative standard deviation calculated on injected standards during one run varied between 0.6–1.2%. For completely dissolved tablets the relative standard deviation was approx. 1%.

In routine work, it is essential that the membrane in the phase separator is durable. This was tested by injecting 20 samples from a completely dissolved felodipine tablet, followed by five injections of standard and repeating 5 times, to make a total of 100 sample injections and 25 standard injections. The test corresponds to two complete dissolution tests comprising six tablets each. The durability test was repeated for three individual membranes. None of the membranes showed any tendency to deteriorate and let water through. Furthermore, two of the dissolution tests were run with the same membrane, without showing any tendency to let through water. Probably the membrane does not have to be changed very often but a daily rinsing with ethanol and chloroform is recommended.

Conclusions

The FIA-extraction system presented is designed for use in routine work. Therefore the low-pressure peristaltic pumps normally used in FIA were substituted for high-pressure LC pumps. Thus there was no need for changing and adjusting pumptubes, an operation that takes some skill and has to be made frequently. Furthermore, LC pumps can be expected to have a better flow stability. As distilled water is used both as the aqueous carrier and to pump chloroform from the displacement bottle, closing and starting procedures are very brief. The daily maintenance was limited to filling the displacement bottle with chloroform and rinsing the separator membrane.

The FIA-extraction has proved to equal the present LC method in precision and accuracy. The FIA-extraction has, however, 5 times the sampling frequency. This means that it is possible to analyse the dissolution baths directly without storing samples. Furthermore, the FIA-extraction could easily be incorporated in a fully automated sampling and analysing system for dissolution testing.

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