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High-performance liquid chromatographic determination of nifedipine and a trace photodegradation product in hospital prescriptions

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Abstract: A HPLC method is developed for the assay of nifedipine and trace amounts of a photodegradation product in pulverized tablets used in the preparation of hospital prescriptions. After liquid–liquid extraction, nifedipine and its photodegradation product are chromatographed using a reversed-phase system which involves UV detection at 254 nm and the use of two internal standards. The trace photodegradation product in pulverized tablets is determined along with nifedipine by the same procedure. The method is capable of determining 0.5–5.0 μg of nifedipine using *p*-nitronifedipine as the internal standard and 0.01–0.5 μg of the photodegradation product using 5-hydroxynifedipine as the internal standard. The standard error is found to not exceed 8.4%. The methods are useful for the study of the disposition or photostability of nifedipine in tablets and pulverized tablets.

Keywords: *HPLC; nifedipine; photodegradation product; pulverized tablet; hospital prescription; double internal standard.*

Introduction

Nifedipine, dimethyl-2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydro-pyridine-3,5-dicarboxylate (Fig. 1), is a calcium-channel blocker, which selectively dilates coronary arteries with little or no effect upon other blood vessels. Therefore, nifedipine is frequently used as a coronary vasodilator in the treatment of angina pectoris. This compound is a sensitive substance which decomposes in day-light to give the 4-(2′-nitrosophenyl)-pyridine homologue (Fig. 1), and under UV radiation to give the oxidation product, 4-(2′-nitrophenyl)-pyridine homologue [1,2]. Several investigations have been carried out on the photostability of nifedipine in organic solvents [3], plasma [4, 5] and the solid state [6].

Nifedipine pulverized tablets for a hospital prescription were prepared for clinical pediatric use because the tablets could not be swallowed by certain patients. Accordingly it is necessary to test the photostability of nifedipine in pulverized tablets under artificial light (i.e. room light) as a storage condition, as part of the quality control assessment of hospital prescriptions [7].

In previous studies, the quantitative determination of nifedipine in organic solvents and biological samples was carried out by gas–liquid chromatography [5] and also by HPLC [3, 4, 6, 7].

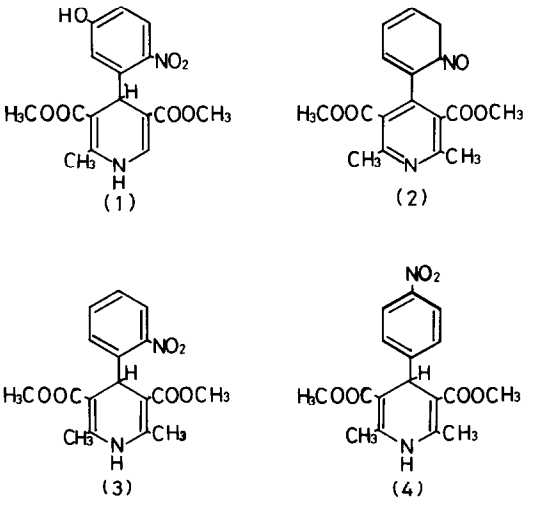

Figure 1
Structure of nifedipine, photodegradation product and internal standards. 1: 5-hydroxynifedipine; 2: photodegradation product; 3: nifedipine; 4: *p*-nitro-nifedipine.

These methods of determination of nifedipine are not suitable for the detection of the early stages of nifedipine photodecomposition and photodegradation processes in hospital prescriptions for samples stored under artificial

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light. Therefore, a highly sensitive detection method for the nifedipine photodegradation product is required.

The detection method needs to be more sensitive for monitoring the increase of the photodegradation product of nifedipine than for monitoring the decrease of nifedipine itself under artificial light conditions. However, the previous methods could not detect trace amounts of photodegradation product in several matrices [3–5].

In the present paper, a simple and selective reversed-phase HPLC method is described which employs two internal standards for the determination of nifedipine, and trace amounts of its photodegradation product in pulverized tablets used in hospital prescriptions.

Experimental

Materials

Nifedipine was obtained by extraction from nifedipine tablets (Adalat L) and purified by recrystallization. The purity was determined and the chemical structure confirmed by a combination of TLC, HPLC, IR and NMR spectroscopy.

The photodegradation product was synthesized as described in the literature [1]. 2,6-Dimethyl-4-(*p*-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate [*p*-nitro-nifedipine] and 2,4-dimethyl-4-(5-hydroxy-*o*-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate [5-hydroxy-nifedipine] (Fig. 1) were also sythesized as described previously [8]. The chemical structures of these compounds were confirmed by IR, NMR and MS.

All the solvents used were of HPLC grade (Wako Pure Chemical Industries Ltd, Tokyo, Japan). Water was distilled in glass and then passed through a 0.45 μm membrane filter (Advantec Inc, Tokyo, Japan). Other reagents used were of analytical grade (Wako Pure Chemical Industries, Ltd, Tokyo, Japan).

Instrumentation

The HPLC system consisted of a Jasco TRI ROTAR-VI solvent pump (Jasco Co. Ltd, Tokyo, Japan), a model VL-614 sample injector, UVIDEC 100-VI ultraviolet detector (operated at 254 nm) and a DS-L300 data module (Jasco Co. Ltd). Samples were chromatographed at room temperature on a 150 × 4.6 mm i.d. column packed with 5-μm Develosil ODS-5 (Nomura Chemical Co. Ltd, Seto, Japan). The mobile phase consisted of 0.01 M disodium hydrogen-phosphate buffer–methanol (45:55, v/v). Before mixing, the buffer was adjusted to pH 6.1 with 50% phosphoric acid. It was degassed ultrasonically and used at a flow rate of 1.0 ml min^{-1}.

Preparation of samples

An aliquot of pulverized tablet was transferred to a centrifuge tube (50 ml) containing 10 ml of water. The mixture was extracted with 10 ml of chloroform on a shaking apparatus for 10 min and centrifuged for 5 min at 2000*g*. The organic layer (0.5 ml) was filtered and transferred to an evaporating flask, and was then evaporated to dryness. The sample was reconstituted with 500 μl of methanol and 10 μl were injected onto the liquid chromatograph.

Preparation of calibration graphs

Stock solutions of nifedipine (2.0 mg ml^{-1}), the photodegradation product (1.0 mg ml^{-1}), *p*-nitro-nifedipine (0.8 mg ml^{-1}) and 5-hydroxy-nifedipine (0.08 mg ml^{-1}) in chloroform were prepared.

Aliquots of various amounts of nifedipine, the photodegradation product (as an analytical sample) and 0.2 mg ml^{-1} of *p*-nitro-nifedipine, and 0.02 mg ml^{-1} of 5-hydroxynifedipine (as internal standards) were taken from these solutions, and were added to the extraction system.

The ratios of the peak height of nifedipine to that of *p*-nitro-nifedipine, and photodegradation product to that of 5-hydroxy-nifedipine were used to construct a calibration graph.

Degradation of nifedipine in pulverized tablets

The breakdown rate of nifedipine in pulverized tablets in normal room light was examined with drug sealed paper.

Pulverized tablets were exposed to laboratory light (a mixture of day-light and fluorescent light) (700 lux) for 0–18 h at 20°C. Initially, and subsequently after 1, 3, 6, 9, 12 and 18 h of exposure, 100 mg samples of the pulverized tablets subjected to the above light conditions, were analysed for nifedipine and the photodegradation product as described above.

Results and Discussion

In the present study, the determination of nifedipine and trace amounts of the photo-

degradation product in the pulverized tablets was performed by the double internal standard method. Two internal standards were selected such that one of the internal standards was eluted before the photodegradation product and the other was eluted after nifedipine. Two standards were considered to be necessary as the retention time of the photodegradation product was close to that of nifedipine. As the result of preliminary experiments, 5-hydroxynifedipine was found to be a convenient internal standard for the photodegradation product whilst *p*-nitronifedipine was the most suitable internal standard for nifedipine.

A typical chromatogram obtained using standard samples is shown in Fig. 2(a). As can be seen 5-hydroxynifedipine, the photodegradation product, nifedipine and *p*-nitro-nifedipine are well separated.

A typical chromatogram of the extract of a photoirradiated and pulverized tablet, to which two internal standards had been added, is shown in Fig. 2(b). No interfering peaks were observed. As can be seen 5-hydroxy-nifedipine, the photodegradation product, was eluted after 5.7 and 7.5 min as adjacent peaks, and then nifedipine and *p*-nitro-nifedipine 10.4 and 15.1 min, respectively, also as adjacent peaks. Therefore, the large nifedipine peak does not interfere with the determination of trace amounts of the photodegradation product in the pulverized tablets.

The calibration plot curve is linear from 0.5 to 5.0 μg in the case of nifedipine and from 0.01 to 0.5 μg in the case of the photodegradation product (Fig. 3). This amply encompasses the early stage on the photodegradation process (0–3 h) for the content of the photodegradation product in the pulverized tablets under weak room light irradiation.

The accuracy and precision of this method for the determination of the nifedipine and photodegradation product were assessed by adding 2, 3 and 5 mg of nifedipine, 0.3 and 0.5 mg of the photodegradation product to the photoirradiated pulverized tablets containing 1.05 mg of nifedipine and 0.18 mg of the photodegradation product, respectively. The recovery values were satisfactory, being within the range 91.2–103.5%. The precision of this method for the pulverized tablets were satisfactory within the range 0.4–8.4%. It may be concluded, therefore, that the HPLC method described in the communication is both highly selective and accurate.

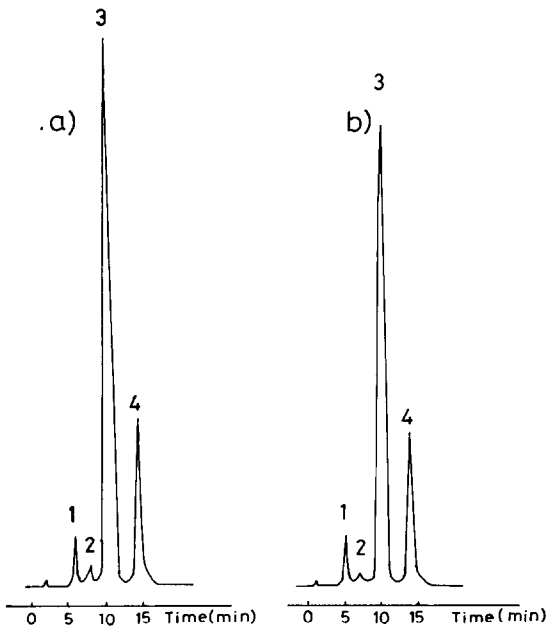

Figure 2
Typical chromatograms of (a) standard sample of nifedipine and photodegradation product with internal standards and (b) photoirradiation sample of pulverized tablet with internal standards. 1: 5-hydroxynifedipine; 2: photodegradation product; 3: nifedipine; 4: *p*-nitro-nifedipine.

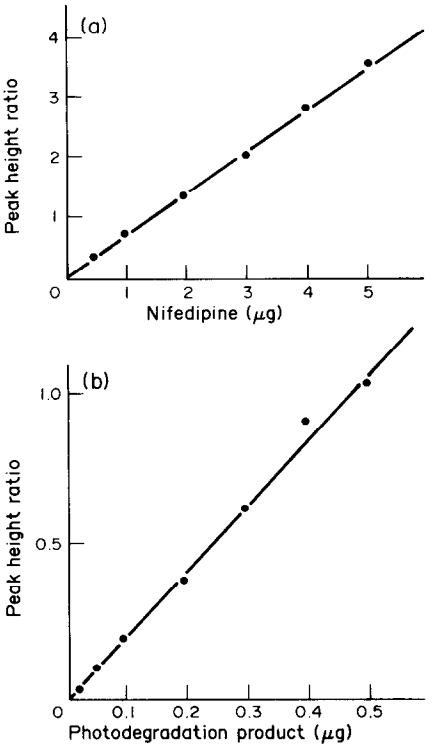

Figure 3
The calibration curve of nifedipine and photodegradation product. (a) nifedipine, (b) photodegradation product.

The photodegradation of nifedipine in pulverized tablet in sealed paper, when used as a hospital prescription is very marked and a decrease of 20% in nifedipine content is observed after 18 h. In addition, an increase in the level of the photodegradation product is observed after 1 h (Fig. 4).

The large variability in photodecomposition rate is due to the storage conditions. There is evidence that the photodecomposition observed in the sealed paper occurred at an early stage of photoirradiation. When determining the level of the photodegradation product by the present method, there was evidence of photodecomposition of nifedipine in less than 1 h in pulverized tablets under the room light irradiation, even when a decrease of the nifedipine concentration could not be detected.

In this paper, the determination of nifedipine and trace amounts of its photodegradation product in pulverized tablets as a hospital prescription is described using a method involving the use of two internal standards. The method enables the measurement of the photodegradation product in the early stage of the photodegradation process which cannot be detected by the decrease of the nifedipine content. The described chromatographic method is also suitable for the quality control of nifedipine in hospital prescription and can be used for pharmacokinetic studies in humans following therapeutic dosages of nifedipine and for monitoring the photodegradation product as a decomposition by-product in hospital prescriptions. Furthermore, it is intended to use the method as an aid to the development of procedures to prevent the photodegradation.

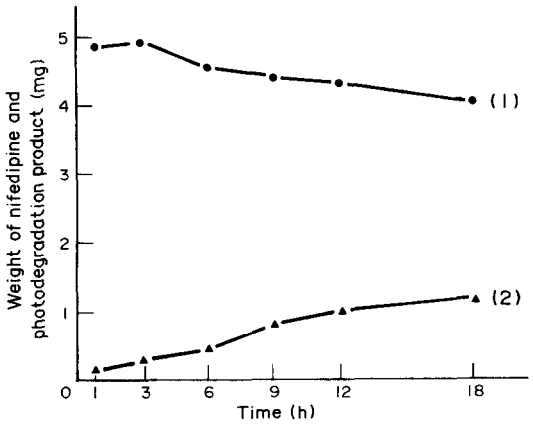

Figure 4
Change of content weight of nifedipine and photodegradation product in pulverized tablet under the room light. 1: nifedipine; 2: photodegradation product.

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