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Short Communication

Analysis of nifedipine in serum using solid-phase extraction and liquid chromatography

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Introduction

Nifedipine (**1**) (Fig. 1) 4-(2′-nitrophenyl)-2,6-dimethyl-3,5-dimethoxy-carbonyl-1,4-dihydropyridine is an anti-anginal and antihypertensive drug, classified pharmacologically as a slow calcium-channel antagonist [1], its pharmacokinetics having been described previously [2, 3]. The assay of nifedipine causes particular problems in that it decomposes in daylight to yield the 4-(2′-nitrosophenyl)pyridine homologue and under UV radiation gives the oxidation product, 4-(2′nitrophenyl)pyridine homologue [4]. Previous methods described utilise organic extraction prior to analysis by high-performance liquid chromatography (HPLC) [4–6], or capillary gas–liquid chromatography [7]. This report describes an LC method using disposable solid-phase extraction columns.

Experimental

Reagents and apparatus

Nifedipine (**1**) was purchased from the Sigma Chemical Co. (Poole, Dorset, UK). The internal standard, SQ 29987 (**2**) was obtained from the Squibb Institute for Medical

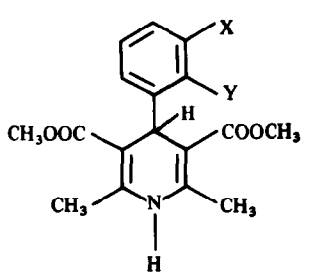

Figure 1
Structure of nifedipine (**1**) X = H, Y = NO_2; and SQ 29987 (**2**) X = NO_2, Y = H.

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Research (Princeton, NJ, USA). All solvents were HPLC-grade from BDH (Poole, Dorset, UK). All the water used throughout this assay was glass distilled. Bond Elut C_{18} disposable 3-ml solid-phase extraction columns were obtained from Jones Chromatography (Hengoed, Mid Glamorgan, UK).

The HPLC system consisted of a Beckman Model 114M pump, operated at a flow rate of 1.0 ml min^{-1}; a Perkin–Elmer (Beaconsfield, UK) Model ISS-100 autosampler with 100-μl sample loop; a Spherisorb C8 5-μm (200 × 4.6 mm, i.d.) reversed-phase HPLC column, which was maintained at ambient temperature; and a Kratos Spectroflow 773 (Applied Biosystems, Warrington, UK) UV detector operated at 235 nm. The mobile phase consisted of methanol and water (65:35, v/v), degassed using helium, prior to use. Amber glassware was used throughout, and all sample manipulation was carried out in subdued lighting to prevent photodegradation.

Standard solutions

A stock solution of nifedipine was prepared in the mobile phase (200 μg ml^{-1}). Dilutions of this solution were made using the eluent to yield solutions containing between 2000–40 ng ml^{-1}. A stock solution of internal standard was prepared in the mobile phase (100 μg ml^{-1}). Dilutions of this stock solution were prepared using the mobile phase to give solutions containing between 500–250 ng ml^{-1}. The working standard prepared daily contained both 40 ng ml^{-1} of nifedipine and 250 ng ml^{-1} of the internal standard and was thoroughly mixed before use.

Sample preparation procedure

To 1 ml of the sample, 50 μl of the 5000 ng ml^{-1} internal standard solution was added. The disposable extraction column was conditioned with one 3-ml wash of methanol followed by two 3-ml washes of water. The sample was passed through the column which was washed with two further 3-ml vol of water to remove endogenous material from the samples. Nifedipine and internal standard were then eluted from the column using 3 ml acetone, and the eluate evaporated to dryness under a gentle stream of nitrogen in a heating block at approximately 50°C. The residue was redissolved in 1 ml of the mobile phase, mixed well and 100-μl samples were injected into the chromatograph.

Recovery

Recovery of nifedipine by the extraction procedure was determined by comparison of the peak height of the standard solution with that of blank serum samples to which nifedipine was added over a range of concentrations.

Results and Discussion

Typical chromatograms for the nifedipine analysis are shown in Fig. 2. Solid-phase extraction of nifedipine from the serum sample was the preferred procedure for the analysis, giving good recovery of nifedipine with no interference from endogenous peaks. Liquid organic extraction of nifedipine from serum with toluene, chloroform, ethyl acetate, hexane and diethyl ether was found to give inadequate recovery and poor reproducibility. Octadecyl phases were used because they quantitatively retained both the nifedipine and the internal standard in the extraction column during the washing of aqueous components from the column. No alternative solid-phase columns were evaluated. Acetone was chosen to elute both nifedipine and internal standard from the

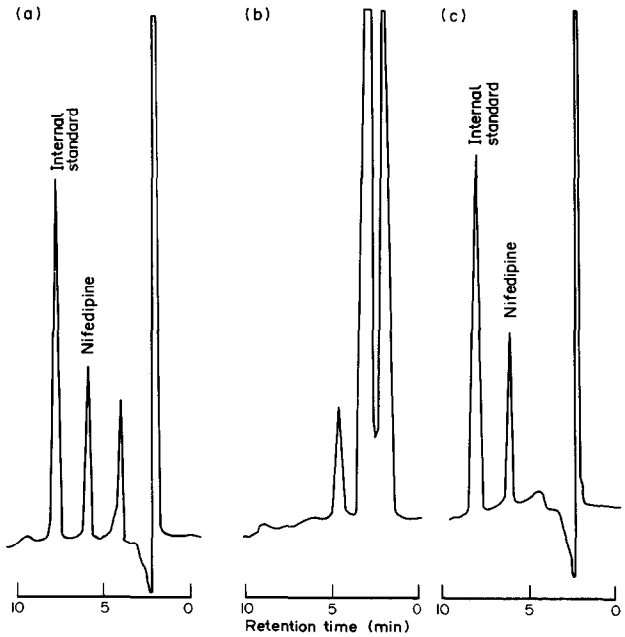

Figure 2
Typical chromatograms from human serum (1 ml) for (a) blank human plasma spiked with 40 ng ml^{-1} nifedipine and 250 ng ml^{-1} internal standard; (b) blank human plasma; and (c) standard nifedipine and internal standard. Detector sensitivity was 0.01 AUFS. Injection volume was 100 μl.

extraction column as both components are very soluble in this solvent which is readily evaporated to dryness.

Linear regression standard curves for the nifedipine standard and the internal standards were constructed over the ranges 0–200 and 0–300 ng ml^{-1}, respectively. A linear response was determined for both compounds (r = 0.9999), with intercepts of -0.12 ± 1.7 and 0.15 ± 2.1 (95% probability) for nifedipine and the internal standard, respectively.

To determine the precision and accuracy of the assay method, replicate samples (2–9) were analysed at five concentrations. The results are summarized in Table 1. The relative standard deviation (RSD) ranged from 0.1 to 5.6 (mean = 2.3). The lower limit of detection was approximately 5 ng ml^{-1} using 1 ml of serum (2 × baseline noise). The absolute recovery of both nifedipine and the internal standard was >95%. The assay could be effectively used without the internal standard; however, its use enables consistent day to day reproducibility (over 51 days mean recovery = 96.7%, SD = 12.7).

There were no detrimental effects to serum samples on storage at −20°C for up to 1 month. Furthermore, it was found that samples could be repeatedly frozen and thawed with no loss of recovered nifedipine. When stored in amber glassware at 4°C there was no loss of nifedipine from the stock solution over a 7-day period. The working standard was found to be stable over several days under conditions described, nevertheless it was prepared daily as a precautionary measure. It has previously been demonstrated that both the daylight and UV radiation photochemical degradation products elute before nifedipine [4], and hence SQ 29987, under reversed-phase HPLC conditions, indicating that the system is specific for both compounds.

Table 1
Precision and accuracy of the nifedipine assay

Concentration of nifedipine (ng ml^{-1})	Recovered nifedipine concentration (ng ml^{-1}), % of theory	RSD%
40	40.0 [100.1%]	0.5
50	48.1 [96.2%]	5.6
75	76.2 [101.5%]	0.1
100	97.0 [97.0%]	3.0
150	145.1 [96.7%]	2.5

The assay of nifedipine in human serum presents many problems. However, the use of solid-phase extraction columns allows rapid and sensitive analysis of samples and has been used to determine the pharmacokinetics at the 40-mg oral daily dosing level.

The use of solid-phase extraction columns enables several samples to be batch processed together which greatly increases the daily sample throughput, from typically 20 samples/day using a liquid–liquid extraction procedure to typically 50 samples/day. This procedure also can be readily automated by using the AASP sample processor (Varian, Surrey, UK) or the Zymate robot [8], which enables a continuous stream of samples to be sequentially prepared and either injected immediately or stored in an autosampler rack for analysis at a later time.

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