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Determination of nifedipine in human plasma by flow-injection tandem mass spectrometry

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Abstract

For use in clinical studies, a fast and sensitive assay method was developed for the determination of nifedipine in human plasma samples. The assay method is based on tandem mass spectrometry detection (HPLC–MS–MS). The effect of flow injection as well as HPLC separation on the results of the nifedipine determination were evaluated. The limit of quantification is 0.5 ng/ml and the accuracy (as determined by spiking recovery) was found to be good.

Keywords: Nifedipine

1. Introduction

Nifedipine (21829-25-4), a 1,4-dihydropyridine calcium antagonist, has been used extensively in cardiovascular diseases (angina, hypertension). For clinical studies, and for pharmacokinetic and bioavailability studies in particular, sensitive assay methods are required. A large number of assay methods have been published, but only a limited number of them have a well-documented lower limit of quantification in the range below 2 ng/ml which is required for such studies [1–3,9].

As part of the running assay development program, a sensitive method based on a GC or GC–MS assay was considered for development. Because of the thermoinstability of nifedipine, special precautions are required to prevent its decomposition in the injection port [4–7].

When reviewing the literature on methods for the determination of nifedipine in plasma samples, it became clear that the use of GC methods using split–splitless injectors could lead to oxidation of the dihydropyridine moiety. As a result of thermal degradation, one of the metabolites of nifedipine, dehydronifepinine, is formed by oxidative dehydrogenation. This problem can be overcome by using on-column injection (and/or adaptation of the temperature of the injection port) [1].

The use of LC–MS–MS was considered to be an alternative for a GC procedure, since no elevated temperatures are required when using this type of instrumentation, operating in the ion-spray mode.

This was one of the reasons why it was decided to explore the possibilities of using HPLC or flow-

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injection with tandem mass spectrometry detection. HPLC–MS–MS methods have been published for other Ca antagonists [3]. To our knowledge, such methods have not been published so far, for the quantitative determination of nifedipine in biological samples. Only one HPLC–MS–MS method is reported for nimodipine, a closely related compound [5].

The photosensitivity of nifedipine has been investigated in detail [1,2,6,8]. Based on these findings, appropriate measures should be taken to avoid degradation of nifedipine due to exposure to daylight and/or artificial light of a special wavelength.

2. Experimental

2.1. Chemicals and reagents

Nifedipine ([21829-25-4], $C_{17}H_{18}N_2O_6$, batch R-144-2) and nitrendipine ([39562-70-4], $C_{18}H_{20}N_2O_6$, batch R47-1, used as internal standard) were provided by Bayer (Wuppertal, Germany). Toluene and formic acid were obtained from Merck (Merck, Darmstadt, Germany); acetonitrile (far-UV quality) was obtained from LabScan (Boom, Meppel, The Netherlands).

2.2. Instrumentation

All experiments are performed on a SCIEX API-III^{plus} triple quadrupole mass spectrometer with the ion-spray interface in the negative-ion mode. The curtain gas flow was 1.2 l/min, the orifice voltage −60 V and collision gas was set at $270*10^{12}$ molecules per cm^2. For flow injection, the Sciex was equipped with a Perkin Elmer Series 200 pump and a Perkin Elmer ISS 200 autosampler. The mobile phase consists of acetonitrile–water–formic acid 950:50:1. The flow, injection volume and run-time were 0.04 ml/min, 20 μl and 2 min, respectively. The scanning dwell-time in the MRM mode was 150 ms and a pause time of 50 ms was used.

Data analysis was performed using the Macquan software (version 1.5) running on an Apple Macintosh Quadra 800 (operating system: Macintosh system 7.5). The following ions are selected for measurement (precursor/product transitions): nifedipine: precursor-ion 345, product-ion 122; nitrendipine: precursor-ion 359, product-ion 122.

For the HPLC separation a short C_{18} column, (Pecosphere, Perkin Elmer part no. 0258–0164, 3.3 cm, 4.6 mm I.D.) was used. For these tests, the Turbo Ionspray was applied with a nitrogen auxiliary flow of 5000 ml/min and temperature at 250°C. The flow-injection volume and run-time were 0.3 ml/min, 5 μl and 2 min respectively.

2.3. Sample preparation

All tubes, vials and flasks in which solutions and samples containing nifedipine are present, were wrapped in aluminium foil and were processed in a darkened room with yellow light (Philips TLD 36W/16 and 15 W Osram 4543).

Plasma samples were thawed, vortexed and centrifuged for 5 min at 3000 rpm. To 1-ml sample aliquots, 2 ml of the internal standard solution (10 ng/ml in toluene) was added. After vortexing, the vials were closed, shaken for 30 min and centrifuged. An aliquot of 1 ml of the toluene layer was transferred to an amber vial. After evaporating the solvent under nitrogen at room temperature, the residue was dissolved in 200 μl of mobile phase.

2.4. Calibration

Calibration standards were prepared at 10 different concentrations in the range of 0.5 to 100 ng/ml. Appropriate amounts of nifedipine stock solutions prepared in toluene were transferred to glass tubes. After evaporation of the solvent at room temperature, plasma was added. Thereafter, the so-prepared calibrators were extracted as described above.

2.5. Cross-validation

Plasma samples (two sets of 36 samples) for cross-validation purposes were prepared by Bayer and provided to BCO. The nominal concentrations were disclosed to BCO only after the results had been reported to Bayer.

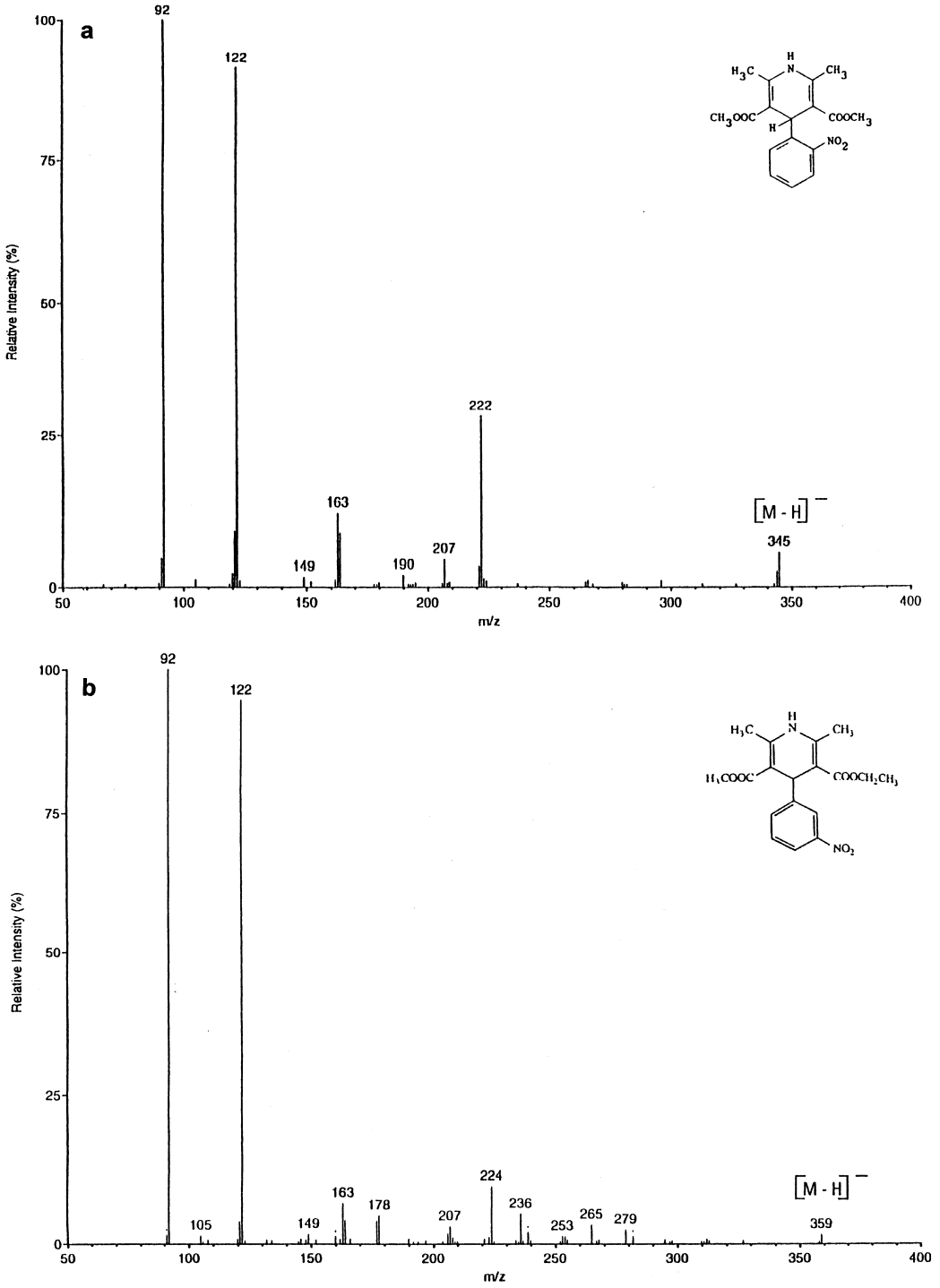

Fig. 1. Product spectra of the precursor parent ion $[M-H]^-$, m/z 345 of nifedipine (a) and the m/z 359 of the internal standard nitrendipine (b).

3. Results and discussion

Product spectra of nifedipine $[M-H]^-$ m/z: 345 and the internal standard nitrendipine $[M-H]^-$ m/z: 359 are shown in Fig. 1a,b.

An example of a calibration graph is given in Fig. 2. The data presented in Figs. 2 and 3 and Table 1 were obtained with the use of the FI–MS–MS equipment. The recalculated concentrations deviated from nominal values less than ten percent over the range of 1–100 ng/ml and within 15 percent at 0.5 ng/ml.

The repeatability and reproducibility data were determined using spiked samples containing 1, 10 and 50 ng/ml. The results are presented in Table 1. Reproducibility data were calculated from the analytical results produced in 6 different runs, the results of the repeatability data set were not included in this calculation. For the samples provided for cross-validation, the repeatability ($n=6$) was also assessed. Within a concentration range from 2.6 up to 91.4 ng/ml, the repeatability, expressed in terms of percentage relative standard deviation (R.S.D.), varies between 4.6 and 5.7%.

Good agreement was observed between the nominal concentrations of the cross-validation samples and the results as obtained with the analytical procedure described above. The correlation between both parameters is demonstrated in Fig. 3. The slope of the correlation curve is 1.018, and the intercept was not statistically different from zero.

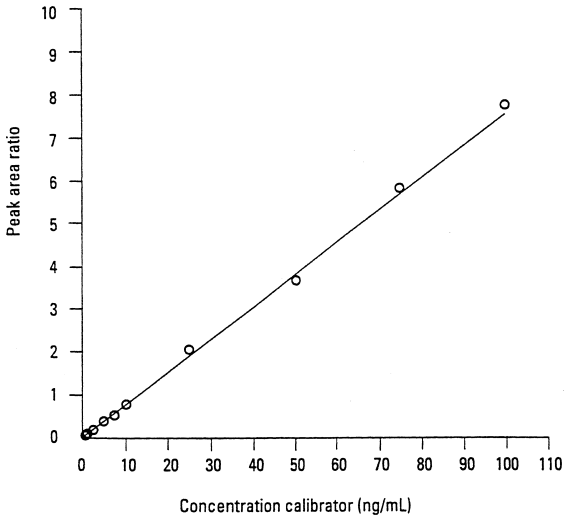

Fig. 2. Calibration curve of nifedipine (peak area ratio=0.075× [nifedipine (ng/ml)]+0.0055; correlation coefficient: 0.998).

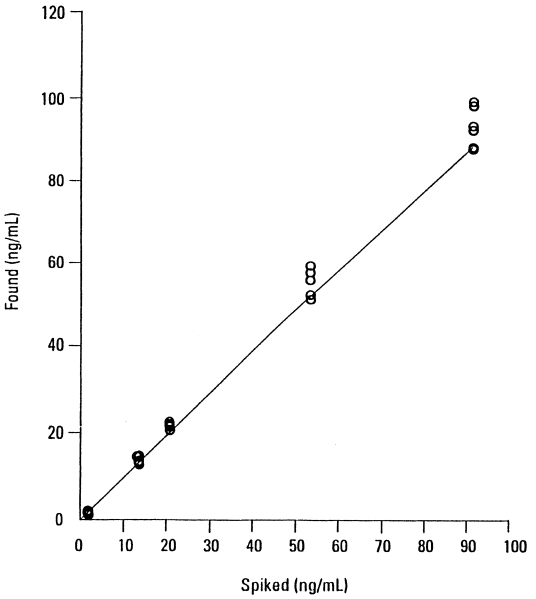

Fig. 3. Relationship between the nominal and the measured concentrations in cross-validation samples (regression coefficient: 1.018; y-axis intercept: −0.397; correlation coefficient: 0.994).

In Fig. 4, the chromatograms of plasma nifedipine and blank plasma are shown registered after flow injection and after an HPLC separation. The chromatograms of the blank plasma do not show any interference after flow injection as well as after an HPLC separation.

Both flow-injection and HPLC separation were evaluated. The advantages of flow injection are short analysis time and simultaneous ionisation of the analyte and the internal standard in the interface. Although, in this case, the retention time of nifedipine on the HPLC column is increased, the overall analysis time is comparable to the flow-injection technique.

Flow-injection analysis with tandem mass spectrometric detection might be considered as critical in the analysis of drugs present in trace amounts in biological matrices. Incomplete or even no chromatographic separation previously might result in ionsuppression in the ionspray, or might introduce interference of endogenous components. Therefore,

Table 1
Repeatability and reproducibility data of the assay ($n=5$)

Concentration (ng/ml)	Repeatability (R.S.D. %)	Reproducibility (R.S.D. %)	Accuracy (%)
1	4.3	12.6	108
10	2.4	4.4	104
50	6.0	2.3	93

the absolute detector response of a standard extracted from plasma was compared with the response of nonextracted standards, dissolved in eluent. The response ratio was 0.87 and 0.85 ($n=10$) for nifedipine and nitrendipine, respectively. Blank plasma extract did show only signal noise as an interference on ion-trace concentrations of the analyte at flow injection and at HPLC separation as well (see Fig. 4). The results presented are all determined by the flow-injection technique.

In order to suppress possible cross-talk effects, caused by the selection of the same product ions for the analyte and the internal standard both, a pause time of 50 ms was introduced. Just for comparison reasons, a short C_{18} HPLC column was introduced. Although chromatographic separation has the advantages of improved peak shapes with corresponding lower detection limits, and less risk of ion suppression in the ionspray and of interference by endogenous components, we proved the selective power of tandem MS with flow injection, also for the determination of trace amounts of drugs in biological matrices. If, however, lower detection limits are required to be achieved, a short HPLC column can be introduced.

The method described combines a high sensitivity with short run-times by using a selective tandem-MS detection mode. When applying the method for the

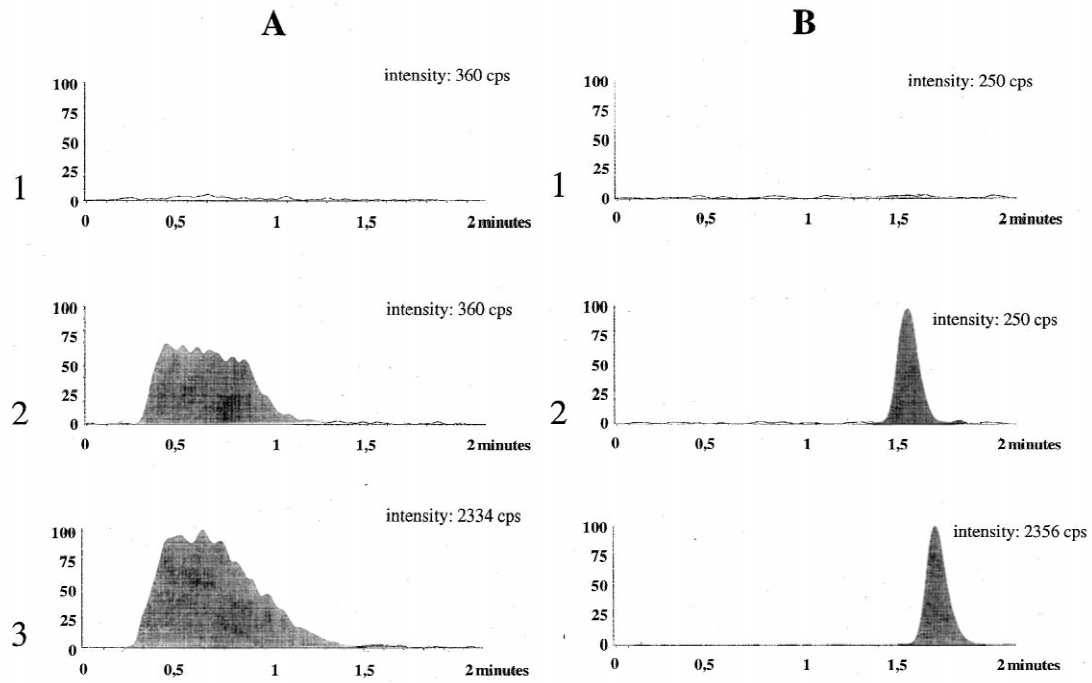

Fig. 4. MRM (multiple reaction monitoring) chromatograms of nifedipine (a) with flow injection and (b) after HPLC column separation: (1) blank plasma, (2) plasma extract (1 ng/ml) of nifedipine and (3) internal standard nitrendipine.

assay of large batches of samples, the sample preparation now becomes the rate-limiting step in the sample throughput chain.

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