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Determination of nifedipine in human serum by gas chromatography–mass spectrometry: validation of the method and its use in bioavailability studies

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Abstract

A procedure for the determination of nifedipine in human serum is described. The light-sensitive substance is isolated from serum by liquid–liquid extraction and analyzed using capillary gas chromatography with a mass-selective detector. The validation of the method shows that the extraction recovery is ca. 85%, the limit of detection is 2 ng/ml and the standard deviations of the intra-day precision test range from 5.8 to 7.4% with respect to the concentration. The procedure is highly selective and sensitive. It is especially suited for bioavailability studies because of its stability and high sampling rate.

1. Introduction

Nifedipine is an established dihydropyridine calcium antagonist used extensively to treat angina and hypertension. Since its half-life in human serum reaches only 2 to 2.5 h [1], a dosage regime of 10 to 20 mg three times daily is recommended [2]. For better patient compliance in continuous therapy, nifedipine sustained-release formulations with dosage intervals of 12 or 24 h are of great interest. Therapeutic drug monitoring of nifedipine under normal conditions is not necessary [3], but for bioavailability studies of such sustained-release formulations, a reliable and sensitive assay of nifedipine in human serum is strongly recommended.

A nifedipine assay using capillary gas chromatography with electron-capture detection has been described [4]. An other assay uses high-performance liquid chromatography with electrochemical detection [5]. A less sensitive but very selective assay uses proton magnetic resonance detection [6]. The procedure using capillary gas chromatography and mass-selective detection with electron-impact ionization (EI) described in this work combines the advantages of high selectivity, sensitivity and stability.

2. Experimental

2.1. Apparatus

A gas chromatograph HP 5890 Series II plus (Hewlett-Packard, Waldbronn, Germany) with an electronic pressure programmer, capillary on-

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column injector and HP 7673 autosampler was used. To prevent photodegradation, the autosampler vials were coated with black varnish (Black Magic vials from ASS-Chem, Bad Homburg, Germany). Samples were injected automatically direct on a retention gap (5 m × 0.250 mm I.D. deactivated uncoated capillary column) and separated on a HP-5-MS capillary column (30 m × 0.250 mm I.D.). The detection was performed with a HP 5972 MSD mass-selective detector (all Hewlett-Packard). Data were collected and analyzed by a Hewlett-Packard DOS ChemStation, software version B.02.02.

2.2. Chemicals

Nifedipine (batch H0040289, 99.9% purity) and diazepam (batch 060381, 99% purity) were purchased from Arzneimittelwerk Dresden GmbH (Dresden, Germany). Toluene, P.A. grade and K_2HPO_4 dry P.A. grade were from Merck (Darmstadt, Germany). Helium, quality 5.6, was purchased from Messer-Grießheim (Magdeburg, Germany). Pooled human serum was a gift from the blood bank of the University Hospital of Magdeburg (Germany).

2.3. Sample collection

All manipulations were carried out in a darkened room with yellow light because of the photosensitivity of nifedipine [7]. Blood samples (ca. 10 ml) were collected into glass tubes. To separate serum from blood cells, the samples were centrifuged for 10 min at 2400 *g*. After centrifugation the serum samples were frozen at −20°C until analysis.

2.4. Sample preparation

All manipulations must be carried out under light protection conditions (see above). In a centrifugation vial (10 mm I.D. with a constriction to 5 mm I.D. in the upper third) a 1-ml volume of serum was mixed with 100 mg K_2HPO_4 until the salt was dissolved. This mixture was extracted with 0.2 ml of a solution of 1 μg/ml diazepam (I.S.) in toluene by gently shaking for 30 min. After centrifugation (2400 *g*) the toluene phase was lifted into the constriction of the vial by carefully adding water to the lower phase. About 0.1 ml of the toluene phase was transferred into microinserts for autosampler vials.

2.5. Calibration and quality control samples

Serum samples containing defined amounts of nifedipine were prepared by mixing a solution of 1 μg/ml nifedipine in water with blank serum. For example, a 50 ng/ml sample is made from 50 μl of the nifedipine solution and 950 μl of serum. The calibration sample concentrations used in this work ranged from 5 to 100 ng/ml. To ensure the stability of the assay over a longer period, a sufficient number of quality control samples with nifedipine concentrations of 10, 50 and 100 ng/ml were prepared and aliquots of these solutions were stored frozen. Both the calibration and the quality control samples were treated with the extraction procedure mentioned above.

2.6. Chromatographic conditions

Oven program: 100°C initial temperature, 40°C/min to 200°C, 1 min constant, 12°C/min to 295°C, 5 min constant. The injector temperature followed the oven temperature. The carrier gas (helium) velocity was set to 30 cm/s and held constant over the run time by the electronic pressure programmer. The GC–MS transfer line temperature was set to 300°C. The detector was working in the selected-ion monitoring mode. From 9 to 11 min run time the ions *m/z* 256, 283 and 284 were monitored, the electron multiplier voltage was 1765 V. From 11 to 12 min the ions *m/z* 329 and 284 were monitored at an electron multiplier voltage of 2165 V.

3. Results and discussion

3.1. Chromatography

Fig. 1 shows typical chromatograms from patient samples and from blank serum. Under the described chromatographic conditions, the

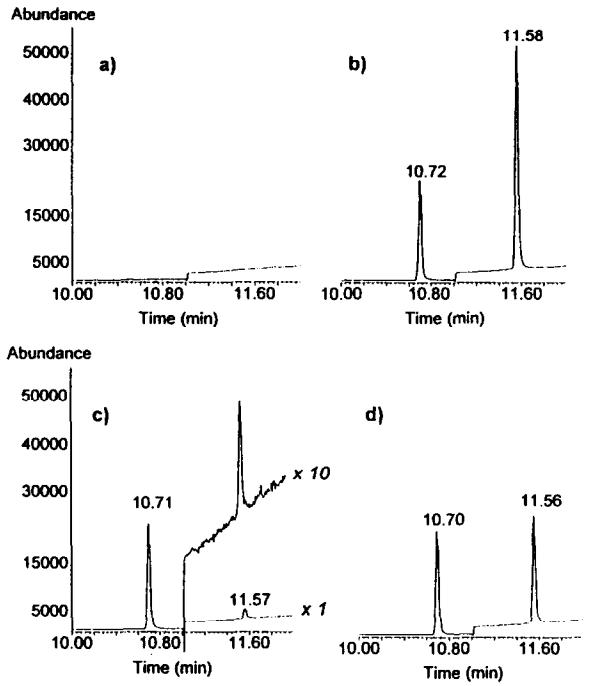

Fig. 1. Representative chromatograms of extracted serum samples. The abundance-axis shows the total of the observed ions (m/z 256, 283 and 284 up to 11.00 min; m/z 284 and 329 from 11.00 min). The I.S. diazepam eluates at 10.7 min, nifedipine at 11.6 min. (a) Blank human serum extract. (b) Blank human serum spiked with I.S. and 100 ng/ml nifedipine. (c) Blank human serum spiked with I.S. and 5 ng/ml nifedipine. (d) Patient sample containing the I.S. and 49 ng/ml nifedipine.

retention times for the I.S. diazepam and for nifedipine were 10.7 and 11.6 min, respectively. The point of discontinuity in the chromatograms at 11.00 min results from the change in the ions monitored and from the rise of the electron multiplier voltage. Although the detector was turned off at 12.00 min, the chromatographic run had to be continued up to 16.5 min, because at 15.8 min a substance, which has been identified as cholesterol, eluted.

The EI mass spectra of diazepam and nifedipine obtained at 70 eV ionisation energy are shown in Fig. 2. A good compromise between selectivity and sensitivity was obtained by monitoring the ions m/z 284, 283 and 256 for diazepam and m/z 329 and 284 for nifedipine. Monitoring these ions, no interfering peaks were observed in the time window of interest. For quantitation, the sum of the peak areas of the corresponding ions was measured.

3.2. Extraction procedure

The yield of the extraction procedure was investigated by multiple measurements of low, medium and high concentrations against toluene solutions, which contained amounts of nifedipine corresponding to 100% yield. The results are summarized in Table 1. The extraction recovery at all three concentration levels was ca. 85%. An unpaired student's t-test showed, that none of the series was significant different from the others. Thus it was concluded that absorption effects do not play a significant role.

In contrast, the extraction time did affect the extraction recovery in an important way. Extraction times less than 30 min resulted in lower recoveries. With extraction times longer than 30 min it was possible to get slightly higher recoveries, but in this case intractable emulsions may be formed. In every day routine it is important to pay attention to a strictly constant extraction time.

3.3. Validation

Selectivity

As shown in the chromatograms (Fig. 1), adequate separation of nifedipine from the I.S. and from endogenous serum substances was achieved.

Concentration–response relationship

In the concentration range from 5 to 100 ng/ml the calibration fit for nifedipine is linear. A calibration sequence of 33 samples with 11 different concentration levels gave a slope of 0.0202 (S.D. 0.00036), an intercept of −0.0171 (S.D. 0.0215) and a regression coefficient of $r = 0.997$.

Limit of detection and quantitation

The limit of detection was 2 ng/ml, where the nifedipine peak is three times higher than the noise level. The limit of quantitation was defined as 5 ng/ml. At this concentration level the R.S.D.s of the peak areas reached 20%, which

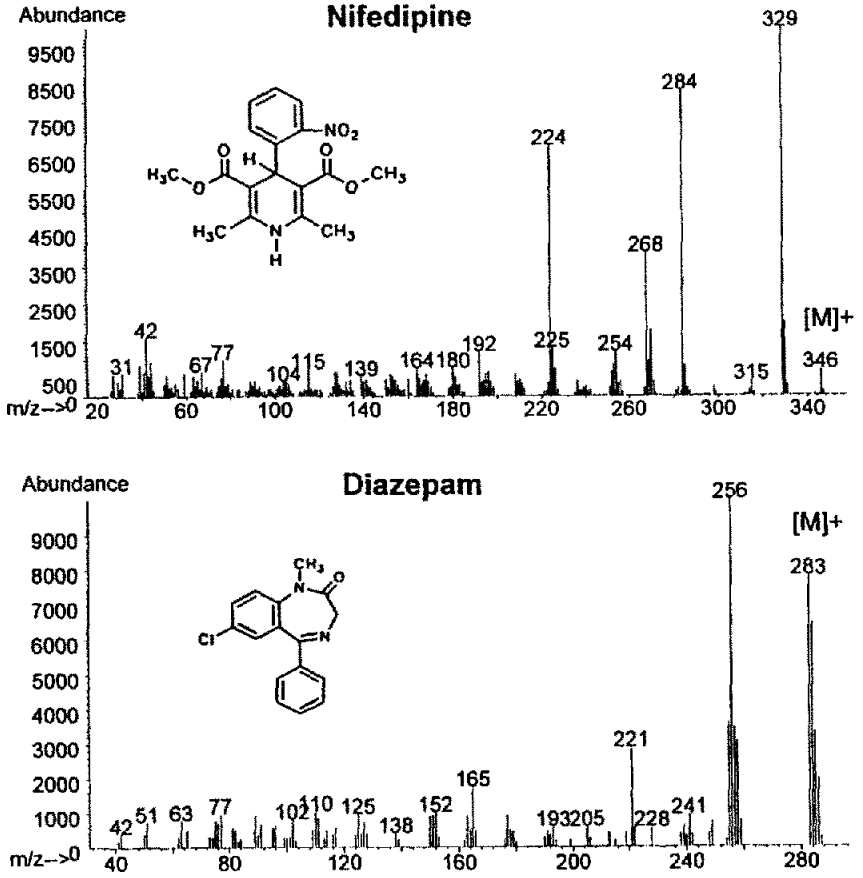

Fig. 2. 70 eV EI mass spectra and molecular structures of nifedipine and the I.S. diazepam.

Table 1
Extraction yields of nifedipine from spiked human serum at three different concentrations

Analysis No.	Peak-area ratio nifedipine/I.S.					
	10 ng/ml		50 ng/ml		100 ng/ml	
	100%	Extracted	100%	Extracted	100%	Extracted
1	0.2764	0.2474	0.9960	0.9062	2.3532	2.1190
2	0.2885	0.2812	1.2198	0.9708	2.4140	1.8610
3	0.3189	0.2508	1.1337	0.8723	2.3359	1.862
4	0.1812	0.1805	1.0078	0.8578	2.3696	2.0131
5	0.2975	0.2826	1.0178	1.0296	2.5992	2.1284
6	0.2522	0.2099	1.0594	0.7781	2.4898	2.0642
Mean	0.2691	0.2421	1.0724	0.9025	2.4270	2.0120
S.D.	0.0484	0.0402	0.0879	0.0885	0.1008	0.1152
Recovery (%)	90.0 ± 18		84.2 ± 10		82.9 ± 6	

was defined as the highest acceptable level for quantitation in our laboratory.

Precision and accuracy

The intra-day precision and accuracy of the assay were tested by multiple measurements of three different concentration levels. The results are summarized in Table 2. As can be seen, the intra-day precision is in the range of 5.8 to 7.4% and the accuracy is in the range of 2.2 to 3.8%. Both the accuracy and the precision are satisfying.

In Fig. 3 the inter-day course of the precision and accuracy measurements are illustrated. The precisions shown in this test are +4.9% for the 100 ng/ml level, +5.0% for the 50 ng/ml level and +8.0% for the 10 ng/ml level. The R.S.D.s are 7.1%, 5.1% and 13.5%, respectively.

Stability under storage conditions

The serum samples were stored frozen at −20°C up to 3 months until measurement. To ensure the stability of the samples under these conditions, test samples were measured after storage under various conditions: freshly prepared, stored one day at room temperature, stored two days at 4°C, twice thawed from −20°C to 25°C and refrozen after 12 h, and stored 3 months at −20°C. Table 3 summarizes the results of these measurements. None of the experiments leads to a result with a deviation exceeding 15% of the spiked value.

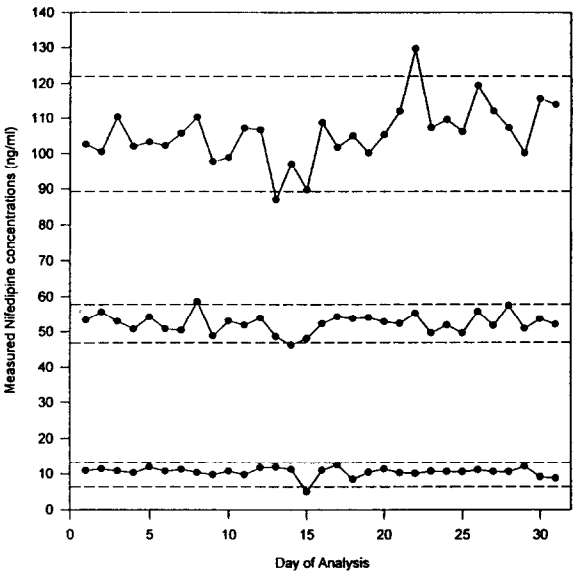

Fig. 3. Measurements of the inter-day precision and accuracy test samples on 31 days over a 3-months period. There are three concentration levels: 10, 50 and 100 ng/ml nifedipine. The dashed lines are indicating the ± 2 times S.D. values of each level. Statistics: $n = 31$; 100 ng/ml: mean = 104.9 ng/ml, R.S.D. = 7.1%; 50 ng/ml: mean = 52.5, R.S.D. = 5.1%; 10 ng/ml: mean = 10.8, R.S.D. = 13.5%.

Quality control in every day analysis

To ensure the accuracy of the results, a series of 6 calibration samples with nifedipine concentrations of 5, 15, 30, 50, 75 and 100 ng/ml was measured every day prior to the batch of

Table 2
Intra-day precision and accuracy data for nifedipine at three different concentrations

No.	Measured concentration of nifedipine (ng/ml)		
	10 ng/ml	50 ng/ml	100 ng/ml
1	9.0	47.5	93.4
2	9.4	50.0	109.6
3	9.6	46.7	104.6
4	10.0	44.2	106.2
5	11.1	50.6	100.6
6	9.6	52.8	108.6
Mean	9.8	48.6	103.8
R.S.D (%)	7.4	6.4	5.8
Accuracy (%)	−2.0	−2.8	3.8

Table 3
Stability of serum samples under different storing conditions ($n = 5$; spiked value 100 ng/ml)

Storing conditions	Concentration found (mean ± S.D.) (ng/ml)
Fresh	100.1 ± 5.20
Two days at 4°C	106.3 ± 5.7
One day at 25°C	102.2 ± 2.10
Freeze–thaw cycle[a]	107.1 ± 7.80
Three months at −20°C	114.4 ± 3.90

[a] From −20°C to +25°C, after 12 h newly frozen to −20°C, two cycles.

unknown samples. Three quality control samples containing 10, 50 and 100 ng/ml, respectively, were measured before and three were measured after the batch of unknown samples to ensure the stability of the assay.

3.4. Application of the method in bioavailability studies

The described method has been used to analyze more than 1500 samples in a bioavailability study of two galenic formulations of nifedipine [8]. The serum sample concentrations normally ranged between 10 and 80 ng/ml. Samples which contained more than 100 ng/ml nifedipine were diluted with blank human serum and were analyzed once more. In Fig. 4 two concentration–time profiles taken from a healthy volunteer over a 24-h period after intake of a dose of 20 mg nifedipine from each galenic formulation are presented.

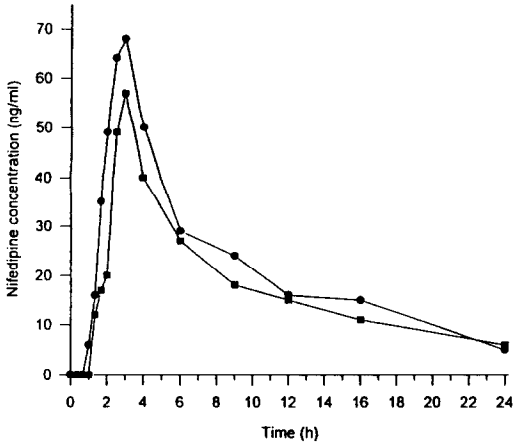

Fig. 4. Concentration–time profile of nifedipine in serum after oral intake of 20 mg nifedipine from two different galenic formulations by a healthy volunteer.

4. Conclusion

The described method presents a sensitive and selective assay for the determination of nifedipine in human serum. It is possible to measure ca. 70 samples per day, including all calibration and quality control samples. The assay is therefore suitable to process large series of samples as occurring in the course of a bioavailability study.

References

[1] A.G. Renwick, D.R. Robertson, B. Macklin, V. Challenor, D.G. Waller and C.F. George, *Br. J. Clin. Pharmacol.*, 25 (1988) 701.
[2] Product Information Procardia, Nifedipine, Pfizer Laboratories, New York, NY, 1992.
[3] R.G. McAllister, G.L. Schloemer and S.R. Hamann, *Am. J. Cardiol.*, 57 (1986) 16.
[4] B.J. Schmid, H.E. Perry and J.R. Idle, *J. Chromatogr.*, 425 (1988) 107.
[5] N.D. Huebert, M. Spedding and K.D. Haegele, *J. Chromatogr.*, 353 (1986) 175.
[6] G.S. Sadana and A.B. Ghogare, *J. Pharm. Sci.*, 80 (1991) 895.
[7] K. Thomas and R. Klimek, *Pharm. Ind.*, 47 (1985) 207.
[8] U. Tröger, J. Martens and F.P. Meyer, *Drug Res.*, in preparation.