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Determination of quinidine, dihydroquinidine, (3*S*)-3-hydroxyquinidine and quinidine N-oxide in plasma and urine by high-performance liquid chromatography

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Abstract

A specific and sensitive method for the quantitation of quinidine, (3*S*)-3-hydroxyquinidine, quinidine N-oxide, and dihydroquinidine in plasma and urine has been developed. The method is based on a single-step, liquid–liquid extraction procedure, followed by isocratic reversed-phase high-performance liquid chromatography, with fluorescence detection. After extraction from 250 μl plasma and 100 μl urine, the limit of determination is 10 n*M* and 25 n*M*, respectively. For the use as standards, commercially available quinidine was purified from dihydroquinidine; quinidine N-oxide was synthesized.

1. Introduction

Quinidine is one of the oldest antiarrhythmic drugs still used in prevention and treatment of atrial flutter or atrial fibrillation, as well as for ventricular and supraventricular arrhythmias.

Quinidine is metabolized by oxidation to several metabolites in the liver, but about 20% is excreted unchanged by the kidneys. It is still derived from the bark of Cinchona trees and commercially available quinidine contains 5–20% of its dihydro analogue, dihydroquinidine, deriving either from the extraction process of Cinchona bark, or the epimerization process of quinine [1]; it is still not known whether dihydroquinidine is also synthesized by biotransformation in vivo.

Quinidine is a very potent inhibitor of a particular drug metabolizing isozyme of cytochrome P450 that now is called CYP2D6 [2–3], but CYP2D6 does not play an important role, if any at all, in the oxidation of quinidine itself [4–6]. The major enzyme catalyzing the 3-hydroxylation and N-oxidation of quinidine in vitro seems to be a P450 isozyme in the CYP3A family [7]. Thus assessment of quinidine metabolism may provide information about the activity of CYP3A isozymes. The relationship between quinidine and specific P450 isozymes has created new interest in the pharmacokinetics of quinidine.

We wished to have available a sensitive and selective HPLC method for the quantitation of quinidine and some of its known and putative

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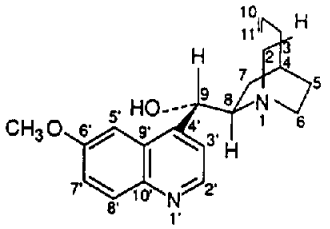
metabolites [(3*S*)-3-hydroxyquinidine, quinidine N-oxide and dihydroquinidine] in plasma and urine of healthy volunteers. For structures see Fig. 1. Compared to previously published methods, the present method generally requires smaller sample volumes [8–11], is more sensitive [8–9,12–13] and is less time consuming regarding analysis time [8–10,14]. This makes the method useful for assessment of quinidine metabolism in large populations of volunteers who are given a very low test dose of the drug.

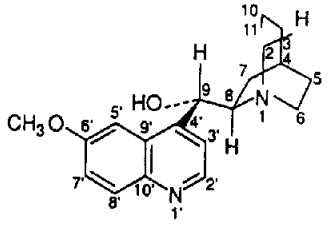
In order to obtain the pure standard, commercially available quinidine was purified from dihydroquinidine. In addition, quinidine N-oxide was synthesized since sufficient quantities could not be procured.

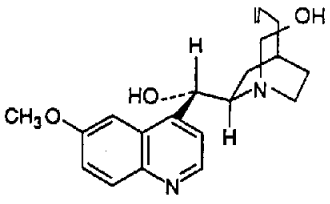
2. Experimental

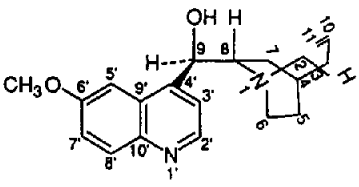
2.1. Chemicals and reagents

Quinidine anhydrous, $M_r = 324.4$ (Sigma No. Q-3625) and Quinine HCl, $M_r = 368.2$ (Sigma No. Q-1125) were purchased from Sigma (St. Louis, MO, USA). (3*S*)-3-hydroxyquinidine free base, $M_r = 340.4$, was kindly supplied by Laboratoire Nativelle (Paris, France). Dihydroquinidine HCl, $M_r = 362.9$ (Aldrich Chemie No. 25,481-9) was purchased from Aldrich Chemie (Steinheim, Germany). Silicagel 60 F_{254} (70–230 mesh), 40% relative humidity, was purchased from Merck (Darmstadt, Germany). Stock solutions for HPLC were prepared









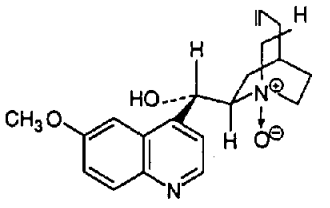


Fig. 1. Structures of quinidine, its major metabolites, quinine and dihydroquinidine.

in methanol and stored at −20°C. Chemicals were of analytical grade and purchased from Merck. Methylene chloride, acetonitrile and methanol were of HPLC grade (LiChrosolv) and obtained from Merck. Sodium hydroxide, sodium perchlorate monohydrate, and perchloric acid 60% were of analytical grade and also obtained from Merck. Water was purified by reversed osmosis and distillation. The samples were eluted with acetonitrile–methanol–sodium perchlorate solution pH 2.5 (6.2:17.3:76.5, v/v). The aqueous sodium perchlorate solution was prepared by addition of 14.05 g of sodium perchlorate and 1.6 ml of 60% perchloric acid to 5 l of water. The aqueous solution was filtered through a Millipore filter (0.45 μm) and the eluent was degassed prior to use.

2.2. Instrumentation for identification

The NMR spectra were obtained on a Bruker AC-250, 250 MHz spectrometer. Electron impact mass spectra (70 eV) were obtained on a Varian MAT 311 A, interfaced to a SS200 data system. Sample temperature was 145°C for quinidine and 135°C for quinidine N-oxide. For HPLC instrumentation, see below.

2.3. Purification of quinidine from dihydroquinidine

The commercially available quinidine was purified from dihydroquinidine by preparative column liquid chromatography, as previously described [15]. The melting point of the dried white crystals was 168°C. The crystals were identified on HPLC, ^{13}C NMR, ^{1}H NMR and mass spectrometry (MS). For the NMR analysis, dimethylsulphoxide (DMSO-d_6) was used as solvent and tetramethylsilane (TMS) as internal standard (μ = 0.00 ppm). The ^{13}C and ^{1}H NMR spectra were in accordance with previously reported data [16,17]. The mass spectrum showed the same fragmentation pattern as previously reported [18].

2.4. Synthesis of quinidine N-oxide

Quinidine N-oxide, M_r = 340.4, was synthesized from the purified quinidine by oxidation with hydrogen peroxide, according to the method described by Guentert et al. [19]. Crystallization of 1.25 g of purified quinidine from ethyl acetate yielded 0.78 g (53%) pure crystals with a m.p. of 147–150°C. The crystals were identified by HPLC, ^{13}C NMR, ^{1}H NMR, and mass spectrometry. The HPLC chromatogram of the synthesized product was compared to a reference sample of quinidine N-oxide synthesized, and kindly supplied by Vanderbilt University Medical Center (Nashville, TN, USA). The ^{13}C NMR and the ^{1}H NMR spectra were obtained in chloroform ($CDCl_3$-d_1), and tetramethylsilane (TMS) was used as internal standard (μ = 0.00 ppm). The ^{13}C NMR spectrum was identical with data reported by Wenkert et al. [16], except for ^{13}C(10) and ^{13}C(11). However, the present chemical shifts of ^{13}C(10) and ^{13}C(11): 116.6 and 137.4 ppm, respectively, were in accordance with reported data by Guentert et al. [19]. Except for the broad singlet at 9.2 ppm, the ^{1}H NMR spectrum was in accordance with reference values [19], and so was the mass spectrum. In order to avoid thermal decomposition [20] of the N-oxide, the spectra were recorded with the lowest feasible sample temperature. At 135°C reproducible spectra were obtained in which the intensity of the molecular ion peak was 84% of the base peak at m/z 323, corresponding to $[M - OH]^+$. The peak at m/z 136, previously reported [19] to be the base peak, had a relative intensity of 19%.

2.5. HPLC instrumentation and conditions

The Hitachi HPLC system (Hitachi, Tokyo Japan) consisted of a Model L-6200 Intelligent Pump, an AS-2000 Autosampler, with a 100 μl injection loop, and an F-1050 Fluorescence Spectrophotometer. The system was controlled through a D-6000 HPLC Interface Module and a personal computer (IBM). The column was a LiChroCART 125-4 packed with 5-μm LiChros-

pher 60 RP-select B (Merck; Cat. 50829). The column was equipped with a guard column LiChroCART 4-4, packed with 5-μm LiChrospher 60 RP-select B (Merck; Cat. 50963).

The elution was carried out at a flow-rate of 1.0 ml/min. The column effluent was quantified at an excitation wavelength of 365 nm and emission wavelength of 415 nm.

2.6. Sample pre-treatment

To 250 μl of plasma (or 100 μl of urine) in a 10-ml glass test tube, 25 μl of 1 *M* NaOH (or 10 μl of 1 *M* NaOH in urine samples), 100 μl of a 10-μM quinine solution in methanol, as an internal standard, and 3.0 ml methylene chloride were added. The mixture was vigorously shaken for 5 min (210 rpm), and centrifuged for 10 min at 1 400 *g*. The aqueous phase was sucked off and the test tube was placed in −50°C in 1–2 min. The organic phase was transferred into a conical glass test tube, placed in a water bath and evaporated to dryness at 50°C. The residue was dissolved in 1 ml mobile phase, vortex-mixed for 10 s and centrifuged for 30 s at 1400 *g*. A 20-μl aliquot was injected onto the column.

3. Results and conclusion

3.1. Selectivity

Base line separation of quinidine, the metabolites, and the internal standard were achieved with the applied conditions (see Fig. 2B and Fig. 3B). About 14 min were required for the analysis. The retention times in minutes were 4.09 for (3*S*)-3-hydroxyquinidine (1), 7.32 for quinidine N-oxide (2), 8.87 for quinidine (3), 10.51 for quinine (4), and 11.99 for dihydroquinidine (5). No interference from impurities produced by the plasma, urine, or the additives from the sample preparation were detected. Representative chromatograms for blank plasma (A), spiked plasma (B), and plasma from a healthy volunteer (C) are shown in Fig. 2. Chromatograms of blank urine (A), spiked urine (B), and urine from the same volunteer (C) are shown in Fig. 3.

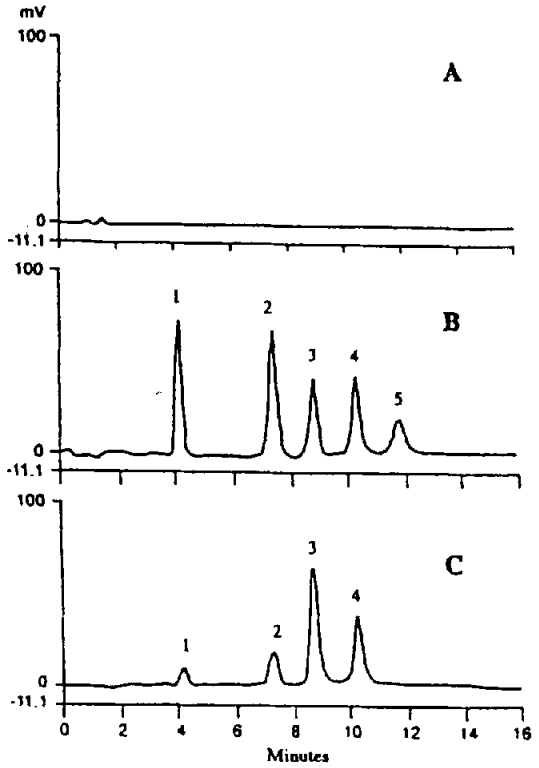


Fig. 2. (A) Chromatogram of blank plasma. (B) Chromatogram of plasma, spiked to 1.5 μ*M* with (3*S*)-3-hydroxyquinidine (1), quinidine N-oxide (2), quinidine (3), quinine (4) and dihydroquinidine (5). (C) Chromatogram of plasma from a healthy volunteer, 2.83 h after a single oral dose of 200 mg purified quinidine, containing 265 n*M* (3*S*)-3-hydroxyquinidine (1), 400 n*M* quinidine N-oxide (2) and 2.765 μ*M* quinidine (3). 100 μl of 10 μ*M* quinine (4) was added as internal standard, prior to extraction.

3.2. Recovery

The absolute recovery of each compound was assessed ($n = 10$) at 5 concentration levels by comparing the peak area after extraction with the peak area obtained from direct injection of equivalent quantities of pure standard. The 5 concentration levels were: 0.25, 0.50, 1.0, 5.0, and 10.0 μ*M* for plasma and 0.25, 1.0, 5.0, 10.0, and 25.0 μ*M* for urine. The recoveries in per cent are shown in Table 1. There was no discernible trend in the concentration and recovery relationship.

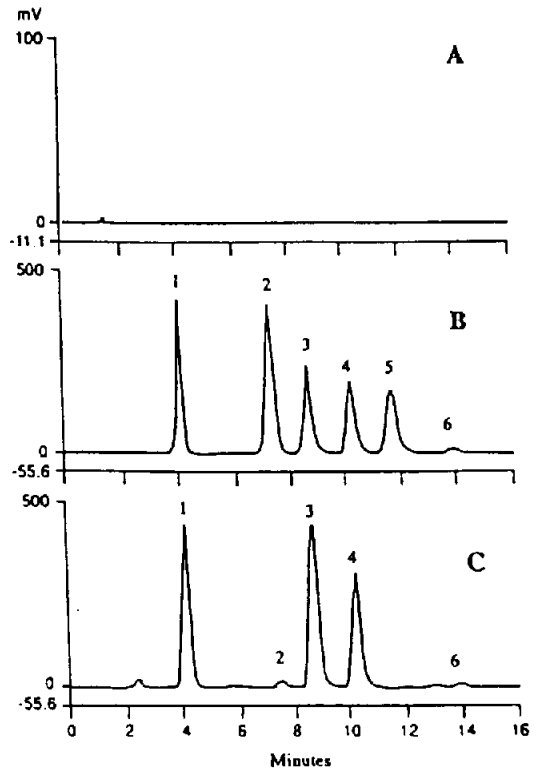


Fig. 3. (A) Chromatogram of blank urine. (B) Chromatogram of urine, spiked to 10 μM with (3*S*)-3-hydroxyquinidine (1), quinidine N-oxide (2), quinidine (3), quinine (4) and dihydroquinidine (5). (C) Chromatogram of urine, from a healthy volunteer, after a single oral dose of 200 mg purified quinidine, containing 14.37 μM (3*S*)-3-hydroxyquinidine (1), 0.54 μM quinidine N-oxide (2) and 31.0 μM quinidine (3); 100 μl of 50 μM quinine (4) was added as internal standard, prior to extraction. Peak (6) is dihydroquinine, an impurity in commercially available quinine.

3.3. Linearity

Linearity of detector response to different concentrations of each compound was determined at plasma concentrations of 0.25, 0.5, 1.0, 5.0, 10.0 μM, and at urine concentrations of 0.25, 1.0, 5.0, 10.0, 25.0 μM for each compound. The standard curves for the five compounds were linear over the investigated concentration range when peak area ratios (compound:internal standard) were plotted against concentration and applied to a least-squares regression equation. Regression correlation data, *y* intercept, slope, and correlation coefficient for each compound are given in Table 2.

Prior to analysis of unknown samples, calibration curves were prepared of three standard levels, covering the expected concentration range. The linear calibration curves were fitted through the data points by linear regression. The quantitative analysis of an unknown sample was derived with reference to the internal standard.

3.4. Repeatability

The intra-day repeatability of the method was evaluated by repeated analysis ($n = 10$) of samples of plasma and urine. Five concentrations of quinidine, (3*S*)-3-hydroxyquinidine, quinidine N-oxide, dihydroquinidine, and quinine were investigated: 0.2 5, 0.5, 1.0, 5.0, 10.0 μM in plasma and 0.25, 1.0, 5.0, 10.0, 25.0 μM in urine. The coefficients of variation are given in Table 3.

3.5. Reproducibility

Inter-day reproducibility was assessed for five following days at three concentrations levels: 0.6, 2.4 and 7.2 μM in plasma and 2.0, 8.0 and 16.0 μM in urine, for quinidine, (3*S*)-3-hydroxyquinidine, quinidine N-oxide, and dihydroquinidine. The coefficients of variation are given in Table 4.

3.6. Limit of determination and detection

The limit of determination based on a signal-to-noise ratio of 10:1 was 10 nM in plasma, and 25 nM in urine, for all compounds. The limit of detection, based on a signal-to-noise ratio of 3:1 was 4 nM for (3*S*)-3-hydroxyquinidine and quinidine N-oxide, and 6 nM for quinidine and dihydroquinidine in plasma. In urine, it was 10, 12, 16 and 20 nM for (3*S*)-3-hydroxyquinidine, quinidine N-oxide, quinidine, and dihydroquinidine, respectively. The limit of determination and detection is based on redissolving the sample residue in 100 μl of mobile phase.

Table 1
Recovery of (3S)-3-hydroxyquinidine, quinidine N-oxide, quinidine, quinine (internal standard), and dihydroquinidine in plasma and urine

Compound		Recovery (%)	
		Plasma	Urine
(3S)-3-Hydroxyquinidine	Mean	80	87
	Range	70–89	75–98
Quinidine N-oxide	Mean	89	101
	Range	83–94	95–105
Quinidine	Mean	83	102
	Range	76–89	91–107
Dihydroquinidine	Mean	80	104
	Range	72–88	97–108
Quinine	Mean	78	91
	Range	75–80	84–94

The analysed concentration levels were 0.25, 0.50, 1.00, 5.00, 10.00 μM in plasma and 0.25, 1.00, 5.00, 10.00, 25.00 μM in urine.

3.7. Accuracy

Plasma and urine spiked with quinidine, dihydroquinidine and the two metabolites, to 0.6, 2.4, 7.2 μM in plasma and 2.0, 8.0, 16.0 μM in urine, were analyzed once a day for five days. The mean estimate and deviation from spiked value are given in Table 4. The determined concentrations of the samples of plasma and urine were in accordance with the spiked value.

Table 2
Linearity of peak area ratio (compound:internal standard), y, versus concentration, x, assessed for (3S)-3-hydroxyquinidine, quinidine N-oxide, quinidine and dihydroquinidine

Compound		Plasma	Urine
(3S)-3-Hydroxyquinidine	y-Intercept	−0.004	0.037
	Slope	0.559	0.227
	r	0.9996	0.9992
Quinidine N-oxide	y-Intercept	−0.056	0.042
	Slope	0.756	0.298
	r	1.000	0.9995
Quinidine	y-Intercept	−0.044	−0.046
	Slope	0.624	0.250
	r	0.9998	0.9999
Dihydroquinidine	y-Intercept	−0.089	−0.045
	Slope	0.667	0.250
	r	0.9998	0.9999

The concentrations were 0.25, 0.50, 1.00, 5.00 and 100.0 μM in plasma and 0.25, 1.00, 5.00, 10.00 and 25.00 μM in urine.

Table 3
Intra-day repeatability of (3*S*)-3-hydroxyquinidine, quinidine N-oxide, quinidine and dihydroquinidine

Compound		Coefficient of variation (%)	
		Plasma	Urine
(3*S*)-3-Hydroxyquinidine	Mean	4.0	3.6
	Range	3.0–5.1	2.2–7.5
Quinidine N-oxide	Mean	3.9	5.4
	Range	3.1–5.7	2.8–10.3
Quinidine	Mean	3.3	5.7
	Range	2.4–4.3	2.9–10.6
Dihydroquinidine	Mean	4.4	6.5
	Range	2.1–8.8	2.3–14.7

Repeatability was assessed by repeated analysis ($n = 10$) of the concentration levels 0.25, 0.50, 1.00, 5.00 and 10.00 μM in plasma and 0.25, 1.00, 5.00, 10.00 and 25.00 μM in urine.

Table 4
Reproducibility and accuracy of (3*S*)-3-hydroxyquinidine, quinidine N-oxide, quinidine and dihydroquinidine

Compound		Plasma			Urine		
		0.60 μmol/l	2.40 μmol/l	7.20 μmol/l	2.00 μmol/l	8.00 μmol/l	16.00 μmol/l
(3*S*)-3-Hydroxyquinidine	Mean	0.63	2.33	8.12	1.88	8.03	16.17
	S.D.	0.02	0.12	0.15	0.06	0.20	0.42
	C.V. (%)	2.9	5.0	1.8	3.3	2.5	2.6
	Deviation (%)	5.1	3.0	12.8	6.1	0.3	1.1
Quinidine N-oxide	Mean	0.59	2.28	7.73	1.90	8.06	16.35
	S.D.	0.03	0.09	0.24	0.14	0.31	0.57
	C.V. (%)	4.3	4.1	3.1	7.2	3.8	3.5
	Deviation (%)	1.2	5.2	7.3	5.0	0.7	2.1
Quinidine	Mean	0.58	2.25	7.66	1.75	7.99	16.07
	S.D.	0.02	0.07	0.16	0.10	0.23	0.23
	C.V. (%)	2.8	3.3	2.1	5.6	2.9	1.5
	Deviation (%)	4.0	6.2	6.3	12.5	2.5	0.4
Dihydroquinidine	Mean	0.57	2.24	7.63	1.70	7.80	15.88
	S.D.	0.02	0.11	0.22	0.06	0.22	0.25
	C.V. (%)	3.0	4.9	2.9	3.5	2.9	1.6
	Deviation (%)	4.7	6.6	5.9	15.1	2.4	0.7

The table shows mean, standard deviation (S.D.), coefficient of variation (C.V.), and deviation from spiked value, in samples from the same pool, analysed once a day for five days.

4. Conclusions

This study describes an isocratic, reversed-phase HPLC method, developed for simultaneous quantitation of quinidine, the two major metabolites (3*S*)-3-hydroxyquinidine, and quinidine N-oxide, and dihydroquinidine in plasma and urine. The method has demonstrated sufficient sensitivity, selectivity and linearity in the applied concentration range. The extraction procedure provides a good overall recovery for all the compounds in plasma and urine. Accurate determination was performed down to 10 n*M* in plasma and 25 n*M* in urine. The one-step extraction procedure and the short analysis time (14 min) thus provide a simple, reproducible and quick assay.

The limit of determination may be further reduced by redissolving the samples, after evaporation, into a smaller volume, e.g. 50 μl of eluent. Therefore, the present method may be convenient for studies of quinidine pharmacokinetics in humans, even in larger populations.

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References

[1] E. Smith, *J. Chromatogr.*, 299 (1984) 233.
[2] S.V. Otton, T. Inaba and W. Kalow, *Life Sci.*, 34 (1984) 73.
[3] R. Brinn, K. Brøsen, L.F. Gram, T. Haghfelt and S.V. Otton, *Br. J. Clin. Pharmacol.*, 22 (1986) 194.
[4] S.V. Otton, R.U. Brinn and L.F. Gram, *Drug Metab. Disp.*, 16 (1988) 15.
[5] G. Mikus, H.R. Ha, S. Vozeh, C. Zekorn, F. Follath and M. Eichelbaum, *Eur. J. Clin. Pharmacol.*, 31 (1986) 69.
[6] K. Brøsen, F. Davidsen and L.F. Gram, *Br. J. Clin. Pharmacol.*, 29 (1990) 248.
[7] F.P. Guengerich, D. Müller-Enoch and I.A. Blair, *Mol. Pharmacol.*, 30 (1986) 287.
[8] M.R. Bonora, T.W. Guentert, A.U. Robert and S. Riegelman, *Clin. Chim. Acta.*, 91 (1979) 277.
[9] T.W. Guentert, P.E. Coates, R.A. Upton, D.L. Combs and S. Riegelman, *J. Chromatogr.*, 162 (1979) 59.
[10] A. Rakhit, M. Kunitani, N.H.G. Holford and S. Riegelman, *Clin. Chem.*, 28 (1982) 1505.
[11] K.A. Thompson, J.J. Murray, I.A. Blair. R.L. Woosley and D.M. Roden, *Clin. Pharmacol. Ther.*, 43 (1988) 636.
[12] R. Leroyer, C. Jarreau and M. Pays, *J. Chromatogr.*, 228 (1982) 366.
[13] G.L. Hoyer, D.C. Clawson and L.A. Brookshier, *J. Chromatogr.*, 572 (1991) 159.
[14] D.E. Drayer, K. Restivo and M.M. Reidenberg, *J. Lab. Clin. Med.*, 90 (1977) 816.
[15] J.M. St-Onge, G. Sirois and M.A. Gagnon, *Eur. J. Drug Metab. Pharmacokin.*, 8 (1983) 363.
[16] E. Wenkert, J.S. Bindra, C.-J. Chang, D.W. Cochran and F.M. Schell, *Alkaloids Acc. Chem. Res.*, 7 (1974) 46.
[17] F.I. Carroll, P. Abraham, K. Gaetano, S.W. Mascarella, R.A. Wohl, J. Lind and K. Petzoldt, *J. Chem. Soc. Perkin Trans.*, 1 (1991) 3017.
[18] K.H. Palmer, B. Martin, B. Gaggett and M.E. Wall, *Biochem. Pharmacol.*, 18 (1969) 1845.
[19] T.W. Guentert, J.J. Daly and S. Riegelman, *Eur. J. Drug Metab. Pharmacokin.*, 7 (1982) 31.
[20] N. Bild and M. Hesse, *Helv. Chim. Acta*, 50 (1967) 1885.