J. Pharm. Pharmacol. 2000, 52: 383–388
Received September 16, 1999
Accepted December 20, 1999

Enhancement of Ursodeoxycholic Acid Bioavailability by Cross-linked Sodium Carboxymethyl Cellulose

SANTO SCALIA, PAOLO GIUNCHEDI*, PAOLO PAZZI† AND UBALDO CONTE‡

*Dipartimento di Scienze Farmaceutiche, Università di Ferrara, via Fossato di Mortara 17, 44100 Ferrara, *Dipartimento di Scienze del Farmaco, Università di Sassari, via Muroni 23/A, 07100 Sassari, †Servizio di Gastroenterologia, Arcispedale S. Anna, Ferrara and ‡Dipartimento di Chimica Farmaceutica, Università di Pavia, via Taramelli 12, 27100 Pavia, Italy*

Abstract

The bioavailability of ursodeoxycholic acid from a new formulation based on drug-loaded cross-linked sodium carboxymethyl cellulose was studied in man. The plasma levels of ursodeoxycholic acid were determined by gas chromatography-mass spectrometry after derivatization and sample purification by solid-phase extraction. Capsules containing the drug/polymer system were prepared and compared with conventional commercial ursodeoxycholic acid capsules after single oral administration using a randomized crossover experimental design.

Although the drug/polymer system improved the in-vitro dissolution rate of ursodeoxycholic acid in simulated intestinal fluid, statistical evaluation of the area under the plasma concentration curves indicated no significant difference in the extent of bioavailability between the two formulations ($14{\cdot}93 \pm 4{\cdot}43$ vs $14{\cdot}95 \pm 5{\cdot}79\ \mu$M h; $P > 0{\cdot}2$). However, following the administration of the ursodeoxycholic acid/cross-linked sodium carboxymethyl cellulose system with an enteric-coated capsule, the mean area under the plasma concentration curve ($27{\cdot}60 \pm 10{\cdot}11\ \mu$M h) was significantly higher than that obtained after treatment with the commercially available ursodeoxycholic acid capsule ($16{\cdot}24 \pm 8{\cdot}38\ \mu$M h; $P < 0{\cdot}05$).

We concluded that improved intestinal absorption of the drug was obtained with enteric-coated capsules filled with the ursodeoxycholic acid/polymer system. Moreover, the simplicity of the preparation and the non-toxicity of the polymer used as the carrier represented additional advantages of this dosage form.

Ursodeoxycholic acid is widely used in capsule or tablet formulations for the treatment of cholesterol gall-stones (Ward et al 1984; Crosignani et al 1996). More recently, the effectiveness of ursodeoxycholic acid in a number of serious liver disorders such as primary biliary cirrhosis, primary sclerosing cholangitis and chronic hepatitis has been reported (Crosignani et al 1996).

In-vivo studies (Parquet et al 1985; Stiehl et al 1990; Crosignani et al 1996) have shown that the bioavailability of ursodeoxycholic acid after oral administration is poor. This unfavourable behaviour has been traced to the low solubility of ursodeoxycholic acid at the physiological intestinal pH (Walker et al 1992) which results in ineffective and dissolution rate-limited drug absorption (Parquet et al 1985; Walker et al 1992; Roda et al 1994).

To increase the bioavailability of ursodeoxycholic acid there is a need for new oral delivery systems with improved dissolution characteristics. Roda et al (1994) have shown that increased intestinal absorption of ursodeoxycholic acid is achieved in man by an enteric-coated formulation containing the drug as sodium salt. Others (Panini et al 1995; Vandelli et al 1995), reported that the inclusion complex of ursodeoxycholic acid with 2-hydroxypropyl-β-cyclodextrin showed enhanced drug aqueous solubility and dissolution rate and improved ursodeoxycholic acid bioavailability after oral administration.

Correspondence: S. Scalia, Dipartimento di Scienze Farmaceutiche, via Fossato di Mortara 17, 44100 Ferrara, Italy.
E-Mail: sls@dns.unife.it

In a previous study (Giunchedi et al 1996), a remarkable improvement of the in-vitro dissolution rate of ursodeoxycholic acid was obtained by using, as drug carriers, water swellable polymers belonging to the group of cellulose and starch derivatives. This study was undertaken to investigate the influence of formulations based on cross-linked sodium carboxymethyl cellulose on the intestinal absorption of ursodeoxycholic acid. Capsules containing the drug/polymer system were prepared and their bioavailability evaluated in healthy volunteers in comparison with commercially available ursodeoxycholic acid capsules.

Materials and Methods

Materials

Ursodeoxycholic acid, trifluoroacetic anhydride and hexafluoroisopropanol were purchased from Sigma (St Louis, MO). 7α-12α-Dihydroxy-5β-cholanic acid was supplied by Calbiochem-Behring (San Diego, CA). Cross-linked sodium carboxymethyl cellulose (CMC-XL; croscarmellose sodium), type A NF was from FMC Corp. (Philadelphia, PA). Cellulose acetate phthalate was supplied by Eastman Kodak (Kingsport, TN). All other chemicals were of analytical grade (Carlo Erba, Milan, Italy). Bond-Elut C_{18} and Bond-Elut SAX cartridges were obtained from Varian (Harbor City, CA).

The conventional ursodeoxycholic acid capsules (Ursofalk; Dr Falk Pharma, Freiburg, Germany) containing 300 mg of ursodeoxycholic acid (capsule excipients were magnesium stearate, starch and precipitated silica) were purchased at a local pharmacy. Their drug content was checked by high-performance liquid chromatography (HPLC) (Scalia 1989).

Preparation of dosage forms

Ursodeoxycholic acid and CMC-XL (1:1, w/w) were milled in a ceramic ball mill for 2 h, at a speed of 30 rev min^{-1}.

Gelatin capsules (Snap Fit 00) were filled with 600 mg of the ursodeoxycholic acid/CMC-XL (1:1, w/w) powder, corresponding to 300 mg of drug.

The enteric-coated formulations were spray-coated using a pan with a solution of the following composition: 5 g of cellulose acetate phthalate, 1·5 g of castor oil, 15 mL of 95% ethanol and acetone to 100 mL. The amount of the cellulose acetate phthalate coating for each capsule was about 63 mg. The enteric coating of the capsules remained unmodified in USP simulated gastric fluid (pH 1·2) for two hours.

The drug content of the examined dosage forms was checked by HPLC (Scalia 1989).

In-vitro dissolution tests

The in-vitro dissolution studies were performed using a modified USP XXII n.2 dissolution test apparatus (Giunchedi et al 1993) at 37°C with a stirring rate of 100 rev min^{-1}. The capsules were placed directly in 5 L of USP simulated intestinal fluid (pH 7·5). At determined time intervals, 3-mL samples of the dissolution medium were withdrawn and replaced with an equal volume of fresh fluid maintained at 37°C. The test samples were filtered (0·22-μm GV-type filters; Millipore, Molsheim, France) and the dissolved ursodeoxycholic acid assayed according to a previously reported HPLC method (Giunchedi et al 1996). Dissolution kinetics for each formulation were determined from the mean of five tests.

Bioavailability study

Six healthy volunteers (4 women and 2 men; 23–42 years; 56–78 kg) with normal liver function tests and without malabsorption syndromes participated in randomized single-dose crossover studies which were approved by the Ethics Committee of the University of Ferrara. Informed consent was obtained from each subject. The test preparation (one capsule containing a quantity of drug/polymer system corresponding to 300 mg of ursodeoxycholic acid) or the reference dosage form (one capsule containing 300 mg of ursodeoxycholic acid) were administered orally to each subject in the morning after a standard hospital meal. The wash-out period between the two formulations was one week. Blood samples were collected just before, and over an 8-h time period after, drug administration. Plasma was separated by centrifugation (3000 *g* for 10 min) and stored at −20°C until required for analysis by gas chromatography-mass spectrometry (GC-MS).

Plasma assay

A portion (1 mL) of the plasma, after thawing and homogenization, was diluted with 4 mL of 0·1 M sodium hydroxide containing 7α-12α-dihydroxy-5β-cholanic acid (1·3 nmol) as the internal standard. The resulting sample was passed through a pre-conditioned (5 mL of methanol and then 5 mL of water) Bond-Elut C_{18} cartridge (sorbent weight,

200 mg). The extraction column was then washed with 10 mL of water and, after light drying by vacuum, ursodeoxycholic acid was recovered by elution with methanol. The methanolic eluent was applied directly to a pre-conditioned (5 mL of methanol, 5 mL of water and then 5 mL of methanol) Bond-Elut SAX cartridge (sorbent weight, 500 mg) collecting the eluent as part of the fraction. An additional 3-mL volume of methanol was passed through the Bond-Elut SAX cartridge to complete the elution of ursodeoxycholic acid. The resulting solution was reduced to dryness in-vacuo and the residue redissolved in 0·5 mL of the derivatization mixture (hexafluoroisopropanol–trifluoroacetic anhydride, 1:2, v/v) as described by Scalia et al (1994). The sample was incubated at 37°C for 60 min, then evaporated under a nitrogen stream, redissolved in 0·5 mL of acetonitrile and subjected to GC-MS.

GC-MS analyses of the hexafluoroisopropyl-trifluoroacetate ester derivative of ursodeoxycholic acid were performed with a GC 8060 gas chromatograph (CE Instruments, Milan, Italy) coupled to an MD 800 mass spectrometer (TermoQuest Italia, Milan, Italy) with transfer line and ion source temperatures maintained at 270°C and 250°C, respectively . The samples (1 μL) were introduced using splitless injection. An OV17 fused silica capillary column (25 m × 0·25 mm i.d.; CE Instruments) was used. The operating conditions were: injector port temperature, 280°C; column temperature, 70°C for 1 min, then programmed at a rate of 20°C min^{-1} to 270°C with a final isothermal period of 3 min; carrier gas (helium) inlet pressure, 70 kPa; ionising electron energy, 70 eV. The MS was operated in the selected-ion monitoring mode scanning m/z 328, 397, 506, 620 with dwell times of 0·1 s. The GC-MS system was controlled by Mass Lab 1·12 software (ThermoQuest). Quantification was on the basis of peak area for the ratio ursodeoxycholic acid/7α-12α-dihydroxy-5β-cholanic acid (internal standard).

Pharmacokinetic analysis

The area under the plasma concentration–time curve (AUC) was calculated with the trapezoidal method using the GraphPad Prism software package (GraphPad Software, Inc., San Diego, CA). The maximum plasma concentration (C_{max}) and the time at which it occurred (T_{max}) were determined by inspection of the plasma concentration–time data. The relative bioavailability (F) of the test formulation was calculated by equation 1.

$$F = (D_{UDCA}/D_{UDCA\text{-}CMC\text{-}XL}) \times (AUC_{UDCA\text{-}CMC\text{-}XL}/AUC_{UDCA}) \times 100 \quad (1)$$

where D_{UDCA}, AUC_{UDCA} and $D_{UDCA\text{-}CMC\text{-}XL}$, $AUC_{UDCA\text{-}CMC\text{-}XL}$ are the doses (D) and the AUC after administration of the capsules containing pure ursodeoxycholic acid or the ursodeoxycholic acid/CMC-XL system, respectively. Statistical analysis was carried out using a paired Student's *t*-test. $P < 0{\cdot}05$ was considered significant.

Results and Discussion

In an earlier investigation (Giunchedi et al 1996), we showed that a marked enhancement of the dissolution rate of ursodeoxycholic acid in simulated intestinal fluid is obtained by milling the drug with cross-linked sodium carboxymethyl cellulose (CMC-XL). To evaluate the influence of this preparation on the in-vivo bioavailability of ursodeoxycholic acid, gelatin capsules containing the drug/CMC-XL (1:1, w/w) system were prepared and their performance was checked initially by in-vitro release studies. Compared with the conventional commercial capsules, a remarkable improvement of the in-vitro ursodeoxycholic acid dissolution kinetics was achieved by the capsule filled with drug-loaded CMC-XL (Figure 1).

The pharmacokinetics of the ursodeoxycholic acid/CMC-XL capsules were studied in comparison with a commercially available preparation. The determination of the bioavailability of ursodeoxycholic acid-containing dosage forms is a

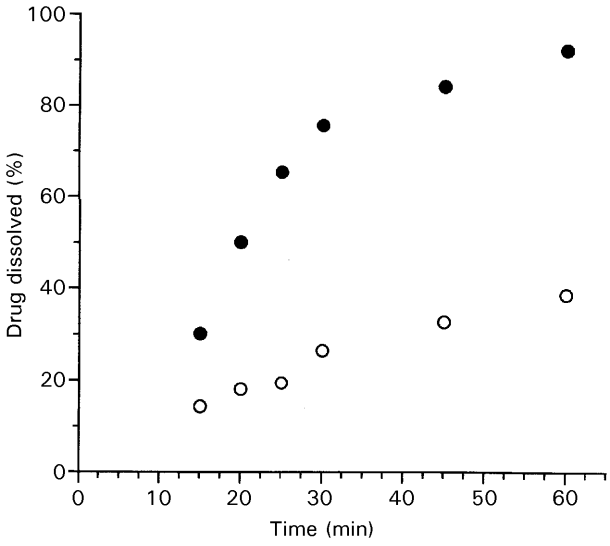

Figure 1. Dissolution profiles, in simulated intestinal fluid, of gelatin capsules containing pure ursodeoxycholic acid (○) or ursodeoxycholic acid/CMC-XL system (●). The relative standard deviation (n = 5) was always < 3·7%.

difficult analytical process because of high first-pass hepatic clearance (Ward et al 1984; Crosignani et al 1996) which results in low drug levels in the systemic circulation (Ward et al 1984; Roda et al 1994; Crosignani et al 1996). Although HPLC enables direct determination of ursodeoxycholic acid, the limited sensitivity of the technique hampers its application to the assay of plasma ursodeoxycholic acid (Roda et al 1988; Scalia 1995). Because of the higher sensitivity attained, gas chromatography coupled with mass spectrometry represents the method of choice (Roda et al 1988; Scalia 1995) for the determination of ursodeoxycholic acid in plasma, following oral administration of the drug. Consequently this technique, combined with preliminary derivatization and sample purification by solid-phase extraction, was selected for the present investigation. The time courses of the mean plasma ursodeoxycholic acid levels after the oral administration of the test (ursodeoxycholic acid/CMC-XL) or the reference (commercially available) capsules are presented in Figure 2. Table 1 summarizes the calculated pharmacokinetic parameters. There was no significant difference in the mean AUC, C_{max} and T_{max} values between the two preparations (Table 1). This indicates a lack of correlation between the in-vitro drug release behaviour (Figure 1) and the in-vivo situation and stresses the importance of validating the in-vitro performance of new formulations by appropriate bioavailability studies. At this point, it seemed reasonable to assume that the observed discrepancy between the in-vitro and in-vivo data could be due to the physiological conditions of the gastric environment wherein the gelatin capsule disintegrated. In fact, the extremely low solubility of ursodeoxycholic acid at the pH value of the gastric juice (Hofmann & Mysels 1992) probably accounts for the inefficient performance of CMC-XL as a drug dissolution rate enhancer in-vivo. To demonstrate this working hypothesis, enteric-coated ursodeoxycholic acid/CMC-XL capsules were prepared with the aim of preventing the dispersion of the drug in the stomach and delivering the ursodeoxycholic acid-loaded polymer to the intestine. The in-vitro dissolution profile, in simulated intestinal fluid, of the ursodeoxycholic acid/CMC-XL system from the enteric-coated capsule was superimposable with that from the gelatin capsule, showing a dissolution greater than 80% after 45 min. The mean plasma concentration versus time curves of ursodeoxycholic acid from the enteric-coated drug/CMC-XL formulation or from the commercially available capsule are presented in Figure 3. The calculated mean values of the pharmacokinetic parameters are listed in Table 2 with the results of statistical analyses. In accordance with previous reports (Roda et al 1994), a pronounced intersubject variability was observed which can be traced to differences in gastric emptying and intestinal motility.

The mean AUC found after administration of the enteric-coated ursodeoxycholic acid/CMC-XL capsule was significantly higher ($P < 0{\cdot}05$) than that achieved by treatment with the commercial capsules. There was no significant difference ($P > 0{\cdot}27$), however, in T_{max} values between the two formulations. Following treatment with the enteric-coated dosage form an increase in mean ursodeoxycholic acid C_{max} was observed (Figure 3 and Table 2), although the difference was not sig-

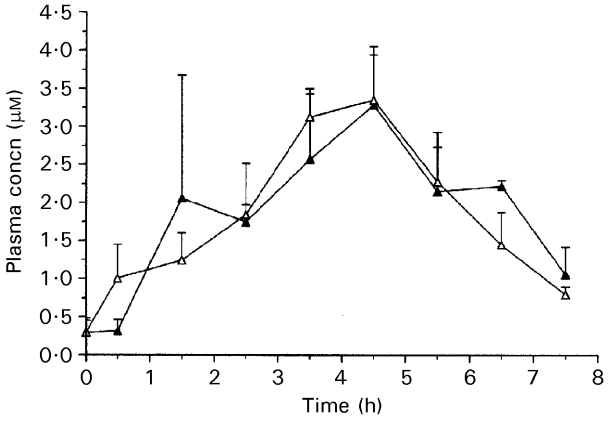

Figure 2. Mean plasma concentration–curves of ursodeoxycholic acid (300 mg dose) for six subjects after administration of a commercial ursodeoxycholic acid capsule (△) or a ursodeoxycholic acid/CMC-XL gelatin capsule (▲). Bars represent s.e.m.

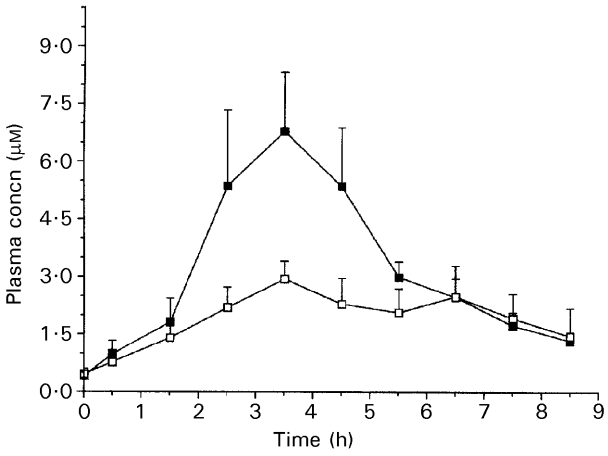

Figure 3. Mean plasma concentration–time curves of ursodeoxycholic acid for six subjects after administration of a 300-mg dose as a commercial ursodeoxycholic acid capsule (□) or an enteric-coated ursodeoxycholic acid/CMC-XL capsule (■). Bars represent s.e.

Table 1. Pharmacokinetic parameters for ursodeoxycholic acid following the administration to 6 subjects of commercial capsules (300 mg of ursodeoxycholic acid) or gelatin capsules containing the ursodeoxycholic acid/CMC-XL system (300 mg of ursodeoxycholic acid).

Parameter	Commercial capsule	Ursodeoxycholic acid/CMC-XL capsule
AUC (μM h)	14·93 ± 4·43	14·95 ± 5·79
T_{max} (h)	4·17 ± 0·58	3·17 ± 1·53
C_{max} (μM)	3·78 ± 0·64	4·68 ± 0·51

Values are expressed as mean ± s.d. Differences between parameters for ursodeoxycholic acid/CMC-XL capsules and commercial capsules were not significant ($P > 0·5$ in all cases).

Table 2. Pharmacokinetic parameters for ursodeoxycholic acid following the administration to 6 subjects of commercial capsules (300 mg of ursodeoxycholic acid) or enteric-coated capsules containing the ursodeoxycholic acid/CMC-XL system (300 mg of ursodeoxycholic acid).

Parameter	Commercial capsule	Ursodeoxycholic acid/CMC-XL enteric-coated capsule
AUC (μM h)	16·24 ± 8·38	27·60 ± 10·11*
T_{max} (h)	4·50 ± 1·41	3·25 ± 0·50
C_{max} (μM)	3·26 ± 1·08	7·23 ± 3·66

Values are expressed as mean ± s.d. *$P < 0·05$, compared with the value for commercial capsules.

nificant ($P = 0·06$). The above results indicate that the improved in-vitro drug release from the ursodeoxycholic acid/CMC-XL system is reproduced in in-vivo conditions provided that an enteric-coated capsule is used. In fact, the novel ursodeoxycholic acid formulation developed in this study, produces a significant enhancement in the extent of drug absorption compared with the commercially available dosage form (Figure 3 and Table 2), as confirmed by the average value for the relative bioavailability (F = 169·95%). Simoni et al (1995) demonstrated that enteric-coated ursodeoxycholic acid capsules do not produce any increase in the AUC with respect to conventional capsules. This indicates that the reason for the effect observed in this study is the CMC-XL polymer and not the gastric protection. Moreover, the higher C_{max} attained (Table 2 and Figure 3) by the enteric-coated ursodeoxycholic acid/CMC-XL capsule is of benefit to hepatocytes (Roda et al 1994). The additional advantages provided by the proposed formulation include the simplicity of its preparation and the nontoxicity of the polymer used as drug carrier.

Other strategies have been described for increasing ursodeoxycholic acid bioavailability from solid oral dosage forms. An enteric-coated formulation of the sodium salt of ursodeoxycholic acid has been reported to achieve higher AUC and C_{max} values compared with a conventional formulation (Roda et al 1994). However, the need for preparation of the sodium salt of the drug is a disadvantage. To enhance the intestinal absorption of ursodeoxycholic acid, a method based on its inclusion in cyclodextrins has also been investigated (Panini et al 1995; Vandelli et al 1995). Following the administration of tablets containing ursodeoxycholic acid complexed with 2-hydroxypropyl-β-cyclodextrin, improved drug bioavailability was attained compared with the commercial preparation, as determined by HPLC-UV. However, given the lack of sensitivity of the HPLC-UV technique (Roda et al 1988; Scalia et al 1994; Scalia 1995), it is surprising that the authors of this study (Panini et al 1995) were able to measure with accuracy the low ursodeoxycholic acid concentrations appearing in plasma during the bioavailability tests. The competitive displacement of the drug from the cyclodextrin cavity by endogenous components, such as other bile acids or cholesterol (Comini et al 1994), limits the in-vivo applicability of the ursodeoxycholic acid/2-hydroxypropyl-β-cyclodextrin complex.

Conclusions

Oral administration of enteric-coated capsules containing the ursodeoxycholic acid/CMC-XL system increases the bioavailability compared with conventional commercially available ursodeoxycholic acid formulations. Since the use of ursodeoxycholic acid-loaded CMC-XL leads to a remarkable improvement in the in-vitro drug release rate, the obtained results support the important role of dissolution in the intestinal absorption process of ursodeoxycholic acid. The enhanced bioavailability achieved by the ursodeoxycholic acid/CMC-XL capsule should lead to higher accumulation of the drug in the enterohepatic circulation and prevent the presence of large amounts of non-absorbed ursodeoxycholic acid and of its biotransformation products (in particular the hepatotoxic lithocholic acid and deoxycholic acid) in the intestine during chronic therapy (Ward et al 1984; Crosignani et al 1996).

References

Comini, S., Olivier, P., Riottot, M., Duhamel, D. (1994) Interaction of β-cyclodextrin with bile acids and their competition with vitamins A and D_3 as determined by ^{1}H-NMR spectrometry. Clin. Chim. Acta 228: 181–194

Crosignani, A., Setchell, K. D., Invernizzi, P., Larghi, A., Rodrigues, C., Podda, M. (1996) Clinical pharmacokinetics of therapeutic bile acids. Clin. Pharmacokinet. 30: 333–358

Giunchedi, P., Maggi, L., Conte, U., La Manna, A. (1993) Linear extended release of a water-insoluble drug, carbamazepine, from erodible matrices. Int. J. Pharm. 94: 15–22

Giunchedi, P., Scalia, S., Maggi, L., Conte, U. (1996) Ursodeoxycholic acid: improvement of dissolution behaviour and its HPLC determination. Int. J. Pharm. 130: 41–47

Hofmann, A. F., Mysels, K. J. (1992) Bile acid solubility and precipitation in vitro and in vivo: the role of conjugation, pH, and Ca^{2+} ions. J. Lipid Res. 33: 617–626

Panini, R., Vandelli, M. A., Forni, F., Pradelli, J. M., Salvioli, G. (1995) Improvement of ursodeoxycholic acid bioavailability by 2-hydroxypropyl-β-cyclodextrin complexation in healthy volunteers. Pharmacol. Res. 31: 205–209

Parquet, M., Metman, E. H., Raizman, A., Rambaud, J. C., Berthaux, N., Infante, R. (1985) Bioavailability, gastrointestinal transit, solubilization and fecal excretion of ursodeoxycholic acid in man. Eur. J. Clin. Invest. 15: 171–178

Roda, A., Roda, E., Festi, D., Colombo, C. (1988) Immunological methods for serum bile acid analysis. In: Setchell, K. D. R., Kritchevsky, D., Nair, P. P. (eds) The Bile Acids. Vol. 4, Plemum Press, New York, p. 270

Roda, A., Roda, E., Marchi, E., Simoni, P., Cerrè C., Pistillo, A., Polimeni, C. (1994) Improved intestinal absorption of an enteric-coated sodium ursodeoxycholate formulation. Pharm. Res. 11: 642–647

Scalia, S. (1989) Determination of bile acids in pharmaceutical dosage forms by HPLC. Anal. Lett. 22: 915–927

Scalia, S. (1995) Bile acid separation. J. Chromatogr. B 671: 299–317

Scalia, S., Cova, U., Fogagnolo, M., Landi S., Medici, A. (1994) Determination of free bile acids in raw materials and bulk products by HPLC and GC. Anal. Lett. 27: 1789–1804

Simoni, P., Cerrè, C., Cipolla, A., Polimeni, C., Pistillo, A., Ceschel, G., Roda, E., Roda, A. (1995) Bioavailability study of a new, sinking, enteric-coated ursodeoxycholic acid formulation. Pharmacol. Res. 31: 1–5

Stiehl, A., Raedsch, R., Rudolph, G. (1990) Acute effects of ursodeoxycholic acid and chenodeoxycholic acid on small intestine absorption of bile acids. Gastroenterology 98: 424–428

Vandelli, M. A., Salvioli, G., Mucci, A., Panini, R., Malmusi, L., Forni, F. (1995) 2-Hydroxypropyl-β-cyclodextrin complexation with ursodeoxycholic acid. Int. J. Pharm. 118: 77–83

Walker, S., Rudolph, G., Raedsch, R. (1992) Intestinal absorption of ursodeoxycholic acid in patients with extrahepatic biliary obstruction and bile drainage. Gastroenterology 102: 810–815

Ward, A., Brogden, R. N., Heel, R. C., Speight, T. M., Avery, G. S. (1984) Ursodeoxycholic acid: a review of its pharmacological properties and therapeutic efficacy. Drugs 27: 95–131