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Assay of free bile acids in pharmaceutical preparations by HPLC with electrochemical detection

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Abstract

A method was developed for the direct analysis of the common free bile acids by high-performance liquid chromatography coupled with electrochemical detection (HPLC-ED). The compounds were separated on an Ultrasphere ODS column with a methanol-acetonitrile-acetate buffer eluent and detected at a porous graphite electrode set at an oxidation potential of +1.4 V. This new technique is applicable to the assay of ursodeoxycholic acid and related impurities in capsule and tablet formulations. Compared with conventional UV detection, the electrochemical detector was found to achieve enhanced specificity and sensitivity.

Keywords: Bile acid; Ursodeoxycholic acid; HPLC; Electrochemical detection; Dosage form

The major bile acids present in humans are cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), lithocholic acid (LCA), and ursodeoxycholic acid (UDCA) which occur primarily as glycine or taurine conjugates (Setchell and Matsui, 1983; Rossi et al., 1987; Scalia, 1990). In addition to their physiological role, two of the foregoing bile acids, namely CDCA and UDCA, have been introduced for the treatment of cholesterol gall-stone diseases (Danziger et al., 1972; Ward et al., 1984). However, because of the side-effects associated with CDCA therapy (Ward et al., 1984), UDCA is the most commonly administered drug (Roda et al., 1993). Furthermore, the introduction of UDCA for the treatment of bile reflux gastritis (Scalia et al., 1988) and especially of cholestatic liver diseases (Poupon et al., 1991) has expanded its use as a therapeutic agent.

Since UDCA is produced from raw materials of animal origin (bovine bile), potential process-related impurities include other bile acids such as the hepatotoxic LCA and DCA and the poorly tolerated CDCA (Roda et al., 1993; Scalia and Games, 1993).

Published procedures for the assay of UDCA in pharmaceutical preparations are based on potentiometry (Campanella et al., 1983), voltammetry (Ferri et al., 1984) and reversed-phase high-performance liquid chromatography (RP-HPLC)

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with UV detection (Scalia et al., 1989). The latter technique is the method of choice for quality control analyses owing to its simplicity, accuracy and precision. However, since the bile acids have weak molar absorptivity, the HPLC technique suffers from limited sensitivity (Roda et al., 1993) and requires the selection of short UV wavelengths (Scalia et al., 1989; Scalia and Games, 1993) which results in increased interference from matrix constituents. HPLC coupled with electrochemical detection (ED) represents a very sensitive technique for the determination of many important analytes. Moreover, enhanced selectivity is achieved by HPLC-ED as a result of the limited number of substances which can undergo redox reactions under certain conditions (Krstulovic et al., 1984). The few reports which have appeared in the literature on the use of ED for the direct HPLC analysis of bile acids have distinct disadvantages, such as the complexity of the apparatus or the procedure (Kemula and Kutner, 1981; Dekker et al., 1991) and the requirement for high-pH (approx. 13) eluents and for laborious electrode maintenance (Dekker et al., 1991). Moreover, the applicability of these techniques to routine analyses of bile acids in their dosage forms was not investigated.

This study describes a novel method for the direct determination of the common free bile acids by HPLC-ED. The application of this procedure to the assay of UDCA and minor related impurities in pharmaceutical preparations and its comparison with conventional UV detection in terms of sensitivity and specificity are also reported.

The sodium salts of CA, CDCA, UDCA, DCA and LCA were obtained from Sigma (St. Louis, MO). Commercial medicinal drugs containing UDCA were purchased at a local pharmacy. Methanol, acetonitrile, water and sodium acetate were HPLC grade from Baker (Phillipsburg, NJ). All other chemicals were of analytical grade (Farmitalia Carlo Erba, Milan, Italy).

The HPLC apparatus (Waters Associates, Milford, MA) comprised two Model 510 pumps, a Model 712 WISP auto-injector and a Model 490E absorbance detector set at 210 nm and 0.02 absorbance units full scale. The UV detector was connected in series with the electrochemical detector (Model 5100A Coulochem; ESA, Bedford, MA) which consisted of a control module and an analytical cell (Model 5010) containing two in-line porous graphite coulometric electrodes operating at oxidizing voltages of +0.60 and +1.40 V, respectively. A single electrode guard cell (Model 5020), set at +1.45 V, was placed between the pump and the injector to suppress background current. The ED sensitivity range and response time were set at 1 μA and 4 s, respectively. Signals from the detectors were integrated by an APCIV computer system (NEC, Boxborough, MA) using Maxima 820 software (Waters). Separations were performed on a 5 μm Ultrasphere ODS column (150 × 4.6 mm i.d.; Beckman, Berkeley, CA) fitted with a guard column (LiChrospher RP-18, 5 μm particles, 4 × 4 mm i.d.; Merck, Darmstadt, Germany) and eluted, isocratically, with methanol-acetonitrile-0.07 M aqueous sodium acetate (55:20:25, v/v) adjusted to pH 5.0 with phosphoric acid. The mobile phase was filtered through GV-type filters (0.22 μm, Millipore, Bedford, MA) and on-line degassed with a Model ERC-3311 solvent degasser (Erma, Tokyo, Japan). Chromatography was performed at ambient temperature, at a flow rate of 0.9 ml/min. The identity of the separated compounds was assigned by co-chromatography with authentic bile acid standards. Peak areas were quantified by the external standardization method.

Individual tablets and capsules were extracted with methanol under sonication, according to the method described in a previous study (Scalia and Games, 1993). Standard solutions in the concentration range 0.05–2.0 mg/ml were prepared by dissolving known amounts of each bile acid reference material in methanol.

Several parameters were examined in order to optimize the electrochemical detection of bile acids. Initial experiments were carried out with a mobile phase consisting of methanol-acetonitrile-0.02 M sodium acetate (pH 4.3), since this eluent has been shown to afford the complete and rapid resolution of the foregoing compounds (Scalia et al., 1989). Under these conditions, bile acids responded at the ED at oxidation potentials higher than +0.8 V. Enhanced signals were obtained as

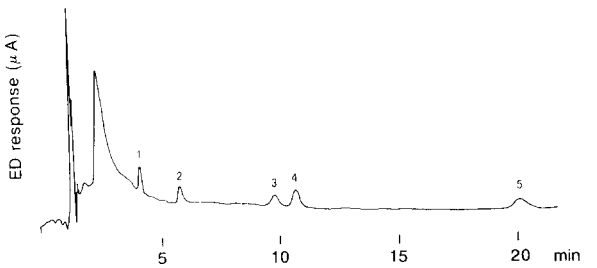

Fig. 1. HPLC separation of a synthetic mixture of free bile acids (approx. 0.5 μg of each component) recorded by ED. Peaks: 1, UDCA; 2, CA; 3, CDCA; 4, DCA; 5, LCA. Operating conditions as described in section 2.

the working electrode potential was increased from +0.8 to +1.4 V. With additional applied potential, no further increase in bile acid peak heights occurred and a rise in the background current was observed; consequently, the operating oxidation voltage was set at +1.4 V. The ED performance was markedly influenced by the ionic strength and pH of the mobile phase. With increasing concentrations of the sodium acetate buffer (from 0.02 to 0.07 M), an increase of the bile acid electrochemical responses was observed in the range 172–214%. Raising the buffer pH from 4.3 to 5.0 produced an average signal enhancement of 102% (range 74–120%). No significant improvement in the detector responses was achieved by further increasing the sodium acetate molarity and pH which were consequently fixed at 0.07 M and 5.0, respectively.

The limit of quantification for bile acids was in the order of 0.20–0.25 μg on-column weight. These values are significantly lower (at least 4-fold) than those reported for conventional UV detectors (Nambara and Goto, 1988; Roda et al., 1993). Calibration curves of peak areas vs amount injected were linear up to 20 μg with correlation coefficients higher than 0.991. A chromatographic recording of a typical separation of a standard mixture of free bile acids (approx. 0.5 μg of each component) obtained with the optimized ED conditions is presented in Fig. 1.

The HPLC-ED system developed in this study was applied to the assay of UDCA and related impurities in pharmaceutical dosage forms. Since the electrochemical detector was coupled on-line with the UV detector, the trace from the ED was monitored in series with the UV trace. Representative chromatograms of UDCA tablet and capsule formulations recorded simultaneously with the two detection systems are reported in Fig. 2 and 3, respectively. Fig. 2 shows that the higher sensitivity achieved by ED (Fig. 2A) compared to UV monitoring (Fig. 2B) permits a more accurate

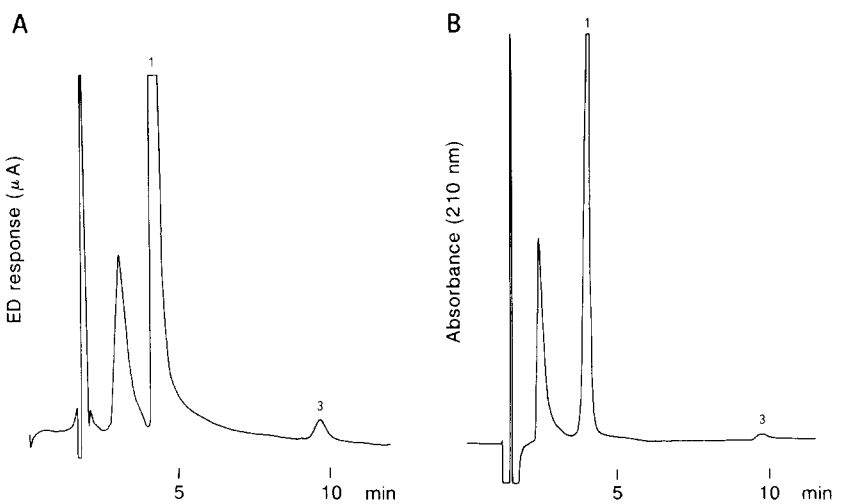

Fig. 2. Chromatographic recordings of a UDCA tablet obtained by: (A) ED or (B) UV detection. Conditions and peak identification as in Fig. 1.

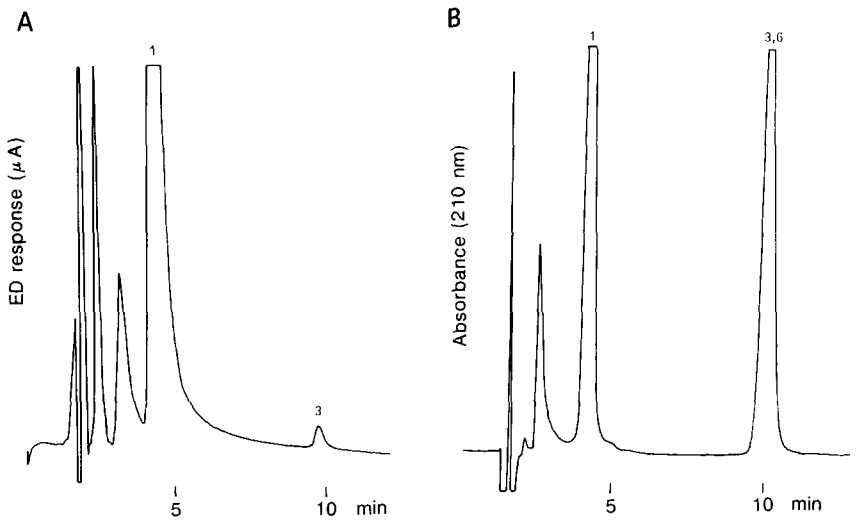

Fig. 3. Chromatographic recordings of a UDCA repeated-release capsule obtained by: (A) ED or (B) UV detection. Conditions and peak identification as in Fig. 1; 6, dibutyl phthalate.

quantification of the trace impurity (i.e., CDCA) present in the UDCA tablet preparation. Moreover, the comparison of the recordings obtained for the same UDCA repeated-release capsule with the two detectors (Fig. 3A and B) demonstrates that the improved specificity of the ED is necessary for quality assurance studies of complex formulations. The large peak interfering with the UV determination of the CDCA impurity (Fig. 3B) and which was traced to one of the capsule excipients (i.e., dibutyl phthalate) was eliminated by electrochemical detection, thus allowing the quantification of the small amount of CDCA present (Fig. 3A).

The response of the ED remained stable over several weeks of continuous use. When the signal intensity declined, the working electrode performance was restored by simply flushing the cell with 6 M nitric acid and/or 2 M sodium hydroxide and then rinsing it with distilled water and methanol.

Five different commercially available UDCA preparations were assayed by HPLC using the proposed ED system (Table 1). No interference was observed in the ED recordings of the placebos. The results presented in Table 1 demonstrate the precision of the method and show compliance with the label claim. Moreover, the present ED technique was compared with classical UV detection on the same pharmaceutical products. Good agreement between the UDCA levels measured with the two detection systems was obtained, whereas the percentage values determined by ED and UV for the minor CDCA

Table 1
Assay results for UDCA and its impurity (CDCA) in individual tablets and capsules determined by HPLC-ED

Pharmaceutical preparation	Label claim	% found [a]	% CDCA [a]
Drug 1 (tablet)	300 mg UDCA	100.2 (1.0)	1.1 (5.2)
Drug 2 (tablet)	150 mg UDCA	103.4 (3.2)	1.3 (3.5)
Drug 3 (capsule)	250 mg UDCA	98.7 (1.8)	1.5 (4.7)
Drug 4 (capsule)	450 mg UDCA	102.9 (2.9)	0.5 (4.9)
Drug 5 (capsule)	300 mg UDCA	101.0 (1.3)	n.d. [b]

[a] Mean (relative standard deviation) of six determinations.
[b] n.d., not detected.

impurity were significantly different. Other potential bile acid impurities (e.g., DCA and LCA) were not detected in the dosage forms.

In conclusion, the ED combined with HPLC offers a means of enhancing the specificity and sensitivity of conventional HPLC-UV analysis of the poorly absorbing bile acids. Because of the high selectivity, good accuracy, reproducibility and sensitivity, the HPLC-ED technique is suitable for quality control assays of commercial medicinal drugs containing UDCA.

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