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The indirect UV detection in the analysis of ursodeoxycholic acid and related compounds by HPCE

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Abstract

A high-performance capillary zone electrophoretic (HPCE) assay has been developed for the determination of ursodeoxycholic acid (UDCA) and its usual impurities. Considering the low molecular absorptivity of UDCA and its related compounds indirect UV detection was used. The electrophoretic capillary was filled with a background electrolyte (BGE) containing an UV absorbing ion: benzoic acid (BA) or 5,5-diethylbarbituric acid (DBA). To enhance the selectivity of the assay diimethyl-β-cyclodextrines (D-β-CDs) or trimethyl-β-cyclodextrines (T-β-CDs) have been added to the running buffer together with methylcellulose or urea. All considered impurities were well resolved with two buffers studied, with the exception of methylursodehoxycholate, a neutral compound. © 1997 Elsevier Science B.V.

Keywords: Capillary zone electrophoresis; Indirect UV detection; Ursodeoxycholic acid; Impurities determination

1. Introduction

In the dissolution therapy of cholesterol gallstones [1] ursodeoxycholic acid (UDCA) is substituted for chenodeoxycholic acid (CDCA) because it is generally well tolerated and does not show the side effects typical of long therapy with CDCA. UDCA, which is the β-epimer of CDCA, has also been found to be a valid drug in other cholestatic liver diseases [2–5]. In UDCA raw material other cholic acids can be present as impurities (Fig. 1); some of them are toxic, like lithocholic acid (LCA) [6,7], or cause many side-effects, like CDCA. The other impurities which can be in the raw material are cholic acid (CA), ursocholic acid (UCA), deoxycholic acid (DCA), methyl ursodeoxycholate (MUDC) and 3-hydroxy-7-chetocholanic acid (HCCA).

Many methods have been suggested to analyze cholic acids like TLC (A. Farina, A. Doldo, V. Cotichini, unpublished data), isotachophoresis [8], electrochemical analysis by using selective sensors [9] and HPLC.

A recent paper by Roda et al. [10] reports a HPLC determination of UDCA and its related compounds comparing the results obtained using

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four different detectors. The detection systems used were fluorescence, light scattering mass detection, refractive index and UV–visable spectrophotometry. Among these detectors, as expected, the UV–visable was, by far, less sensitive. In fact the lack of chromophores in the cholic acids structure gives a very low molar absorptivity which makes the determination of the impurities difficult. We can make up for the lack of molecular absoptivity by utilizing indirect UV detection [11]. If a suitable absorbing ion is chosen as the background electrolyte (BGE) the sensitivity is much enhanced. In fact when the zone, containing the non absorbing analytes reaches the detector, it will displace a defined number of absorbing ions and a decrease in light absorption will occur. The decrease in the absorptivity of BGE will be seen as a negative peak.

For the purpose of having a selective and sensitive assay for quality control of UDCA by high-performance capillary zone electrophoretic (HPCE) using an indirect UV detector, many absorbing ions as BGE have been tested. Among them, benzoic acid (BA) and 5,5-diethylbarbituric acid (DBA) were the best. Dimethyl-β-cyclodextrines (D-β-CD) and trimethyl-β-cyclodextrines (T-β-CD) were used as buffer additives for the purpose of improving the selectivity of the analysis.

2. Experimental

2.1. Apparatus

The electrophoretic experiments were carried out with a Spectra PHORESIS 1000 apparatus (Thermo Separation Products, Fremont, CA) equipped with a multiwavelength UV-visable detector SpectraFocus with deuterium lamp and cooling air circulation by the Peltier effect system (15–60°C).

The capillary electrophoresis was controlled and the data were evaluated using SpectraPhoresis CE ver. 1.05B Software (Thermo Separation Products).

2.2. Chemicals

Pure standards of cholic acids, BA and DBA, methylcellulose and urea were all obtained from Sigma (Milan, Italy), while UDCA raw materials was found locally. Cyclodextrines were obtained from FDS Publications (P.O. Box 41, Trowbridge, Wilts BA 14 8 UE, UK). All other chemicals were of analytical grade and were obtained from Merk (Darmstadt, Germany)

2.3. Electrophoretic conditions

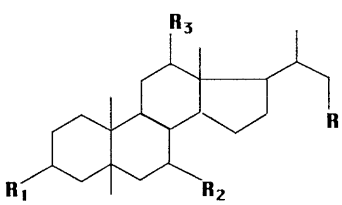
The analytes separation was performed in a bare fused silica capillary (uncoated) with a total length of 50 cm (42 cm effective length) and 50 μm i.d. The analyses conditions were:

BGE I: 50 mM benzoic acid and 100 mM tris hydroxymethyl aminomethane (Tris) at 8.6 pH added to 0.01% methylcellulose and 0.5% T-β-CD or D-β-CD.

Working temperature 20°C.

UV detection 250 nm.

BGE II: 50 mM 5,5-diethylbarbituric acid and 150 mM Tris at 8.9 pH added to 1 M urea and 0.025% D-β-CD or T-β-CD.



R	R1	R2	R3	
CH_3COOH	OH (α)	OH (α)	OH (α)	CA
CH_3COOH	OH (α)	OH (α)	H	CDCA
CH_3COOH	OH (α)	OH (β)	H	UDCA
CH_3COOH	OH (α)	H	H	LCA
CH_3COOH	OH (α)	OH (β)	OH (α)	UCA
CH_3COOH	OH (α)	H	OH (α)	DCA
CH_3COOH	OH (α)	O	H	HCCA

Fig. 1. Chemical structures of UDCA and related compounds.

Working temperature 15°C.
UV detection 236 nm.
Sample loading was made by hydrodynamic mode in a time range 0.5–3 s.
Applied voltage 20 KV (35.4 μA).

2.4. Capillary electrophoresis procedure

Methanolic solutions of pure standards were used to verify the suitability of the two absorbing ions and the other electrophoretic conditions. The best separation conditions were checked with a standards methanolic solution containing 1 mg ml^{-1} of drug added to 1×10^{-2} mg ml^{-1} of each impurity. The linearity of the UDCA calibration curve was verified with methanolic solutions containing different amount of ursodeoxycholic acid, from 0.2 to 1.2 mg ml^{-1}, all added to the same amount (1 mg ml^{-1}) CA, used as internal standard. CA was used to determine UDCA while the concentration of each impurity was obtained from the relationship between the drug and impurities areas. Normalization of the peak area with respect to migration time improved on quantitative results reproducibility.

2.5. Sample preparation

About 10 mg of four different bulk samples of UDCA and two samples of ursodeoxycholic acid bis-emisuccinate (UDC-bis-E) were dissolved in a 10 ml volumetric flask with methanol. These solutions were used for the control of the percentage of impurities in the sample examined.

3. Results and discussion

The determination of UDCA impurities is necessary as some of them are toxic, but all these compounds have a very low molar absorptivity which makes their determination by HPLC or CE with direct UV detection difficult. However, it is possible to increase the detection sensitivity of the CE method by filling the capillary with a very absorbing ion solution. This allows the determination of the variation of the buffer absorbance value caused by the analytes. In fact when the zones of non absorbing ionic species (i.e. UDCA and impurities) reach the detector, they displace a precise amount of the absorbing ion from the BGE. This displacement causes a decreasing in the absorbance value which is shown by a negative peak in the electropherogram. The analysis sensitivity increases remarkably with respect to direct UV detection (λ 190 nm) because it is related to the decreasing absorbance value of the BGE. Using indirect UV detection the highest sensitivity is generally obtained when the effective electrophoretic mobility of the absorbing BGE ion is close to the mobility of the analyte [12] and used at low concentration. Moreover, the similar mobility between analyte ions and background electrolytes allows us to obtain reasonably simmetric peaks. This aspect is important for the quantitative determination of the analytes.

Among many absorbing compounds studied benzoic acid (BA) and 5,5-diethylbarbituric acid (DBA) were the best and they showed an electrophoretic mobility similar to that of the cholic acids studied. Comparing the sensitivity obtained by direct versus indirect UV detection, an increase of about 100 times was obtained with both absorbing ions, even if the sensitivity obtained with DBA was little better.

The impurities in UDCA are many and have molecular structures very close to that of the active compound (some of them are positional isomers) and ,therefore, they need not only high sensitive, but also a very selective analytical method. Because of the similarity of these molecules the two buffer systems were not able to resolve UDCA from its impurities which all comigrate with the active compound. Therefore, D-β-CD or T-β-CD were added to the buffer (Fig. 2a and b, Fig. 3a and b). As it can be seen in the figures the best results were been obtained by adding D-β-CD to DBA whilst T-β-CD was more useful using BA as buffer. These differences in the selectivity are probably due not only to the analytes–cyclodextrines complexation, but also to a possible interaction between BA, or DBA, and cyclodextrines. A further increase in selectivity, using BA, was obtained by increasing the viscosity of the running buffer by adding 0.01% methylcellulose.

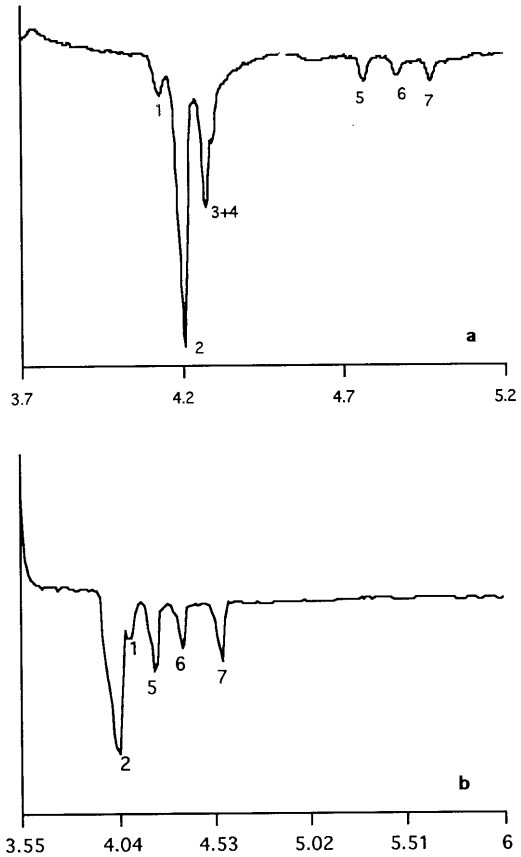


Fig. 2. Working standard solutions: (a) 1 mg ml^{-1} UDCA (2) containing 0.01 mg ml^{-1} LCA (1), CDCA (3), HCCA (4), UCA (5), DCA (6), CA (7). The solution has been enriched with CDCA. BGE I containing 0.5% T-β-CD. (b) 1 mg ml^{-1} UDCA (2) containing 0.1 mg ml^{-1} LCA (1), UCA (5), DCA (6), CA (7). BGE I containing 0.5% of D-β-CD.

The influence of the buffer's cation type and of the ionic strength on the selectivity has been studied. In fact a decreasing in resolution occurs when BA or DBA is titrated with NaOH, while a clear improvement occurs when Tris is used in the buffer titration. Furthermore, concentration of less than 50 mM of either DBA or BA is insufficient to resolve UDCA and its impurities.

Often UDCA is marketed as UDC-bis-E. This compound has, apart from the impurities of UDCA citated above, two other possible impurities, ursodeoxycholic mono-emisuccinate (UDCE) and UDCA. The proposed method also allows the quality control of these compounds (Fig. 4).

The limit of each impurity detection (LOD) was about 0.03 ng nl^{-1} ($S/N = 3$) (Fig. 5), while the limit of quantitation (LOQ) was 0.09 ng nl^{-1} with an R.S.D. = 1.5%.

In the four UDCA bulk materials analyzed only two impurities were found, CDCA (medium value = 0.8%) and LCA (medium value = 1.3%), while in the two samples of UDC-bis-E significant amounts of UDCA (medium value = 0.94%) and UDCE (medium value = 1.1%) have also been found.

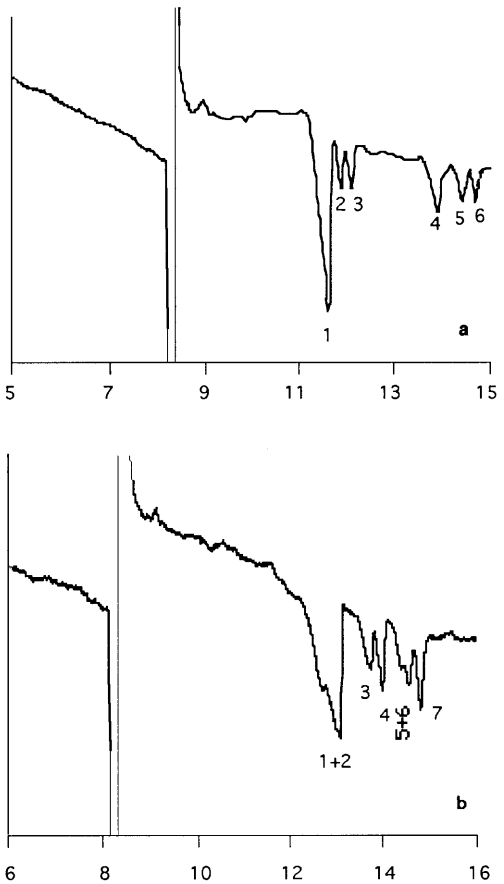


Fig. 3. Working standard solutions: (a) 1 mg ml^{-1} of UDCA (1) containing 0.1 mg ml^{-1} of HCCA (2) CDCA (3), UCA (4), DCA (5), CA (6). BGE II containing 0.025% D-β-CD. (b) 1 mg ml^{-1} UDCA (2) containing 0.1 mg ml^{-1} LCA (1), HCCA (3) CDCA (4) UCA (5), DCA (6), CA (7). BGE II containing 0.025% T-β-CD.

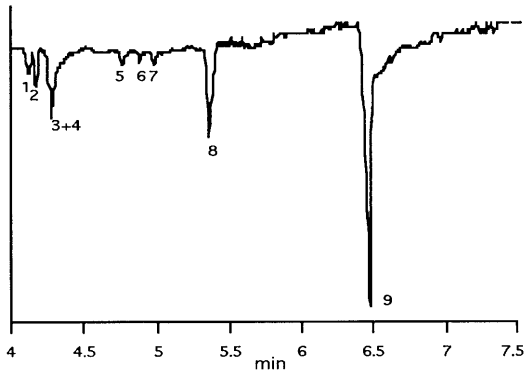


Fig. 4. Separation of UDC-bis-E (9) and its impurities: LCA (1), UDCA (2), CDCA (3), HCCA (4), UCA (5), DCA (6), CA (7), UDCE (8). BGE and electrophoretic conditions as in Fig. 2a.

4. Conclusions

The described HPCE method was developed for assay and purity control of UDCA in raw materials or pharmaceutical form. The indirect UV detection, obtained by adding two absorbing ions, BA or DBA, to the BGE increased the analysis sensitivity by about 100 times allowing us to determine low concentrations of impurities. A small difference in the separation power between BA and DBA has been noted. In fact BA in the BGE allowed a good separation of the drug from all impurities studied in about 8 min, with the exception of MUDC, a neutral compound which coelutes with the elctroosmotic flow. Instead DBA required an analysis time of about 16 min and didn't resolve LCA and the neutral compound.

The determination of impurities by considering the relationship between the UDCA peak area and impurities areas was found to be suitable. In fact it can suppose that UDCA and all impurities studied displace the same number of buffer molecules because their molecular structures are very close to that of the active compound. Furthermore, the normalization of the peak area with respect to migration time improved quantitative results reproducibility. This was confirmed by injecting the same amount of UDCA and impurities and seeing that the peak area values, obtained from each compound, were comparable.

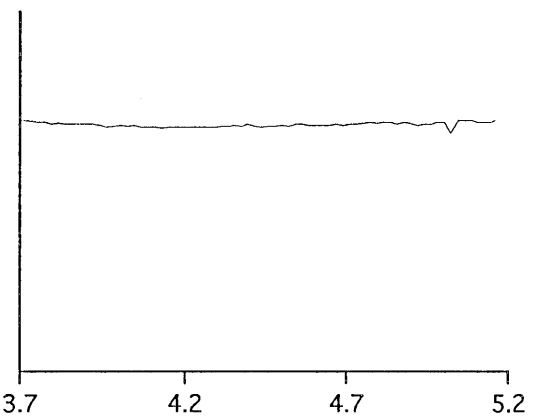


Fig. 5. LOD of CA 0.03 ng nl^{-1}.

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