RPS Pharmacy and Pharmacology Reports, 2022, **2**, 1–5
https://doi.org/10.1093/rpsppr/rqac006
Advance access publication 1 December 2022
Research Paper

RPS Pharmacy and Pharmacology Reports

OXFORD

Dissolution testing of ursodeoxycholic acid suspension using SPE as sample preparation

Oriana Boscolo[1,2,3], Sabrina Flor[1,2,3], Cecilia Dobrecky[1,2], Valeria Tripodi[1,2,3] and Silvia Lucangioli[1,2,3,*]

[1]Departamento de Tecnología Farmacéutica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina
[2]Instituto de Tecnología Farmacéutica y Biofarmacia (InTecFyB), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina
[3]Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Buenos Aires, Argentina

[*]Correspondence: Departamento de Tecnología Farmacéutica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Tecnología Farmacéutica, Junín 956, CP 1113, Buenos Aires, Argentina. Tel: +54 011 5287 4632; Email: slucangi@ffyb.uba.ar

ABSTRACT

Objectives: To develop and validate an analytical method by HPLC–UV (High Performance Liquid Chromatography–Ultraviolet) for the quantification of ursodeoxycholic acid suspension in a dissolution test followed by a solid phase extraction (SPE) to circumvent the interference of sodium lauryl sulphate (SLS) present in the dissolution medium.

Methods: United States Pharmacopeia (USP) apparatus 2. The dissolution medium was 900 ml of an aqueous solution of 0.05 M phosphate buffer (pH 8.4) with 2% SLS. The samples were filtered and cleaned by SPE with 500 mg/3ml C18 cartridges. The analytical method was validated for specificity, linearity, LOD (limit of detection), LOQ (limit of quantification) accuracy and precision. Chromatographic conditions, Symmetry-C18 column (150 mm × 4.6 mm, id; 5 µm particle size), 40°C, 100 µl injection volume and UV detection at 200 nm. The flow rate was 1 ml/min using acetonitrile–phosphoric acid (pH 3.0, 0.15 mM) (48:52).

Key findings: SPE provided an efficient and selective extraction of ursodeoxycholic acid from the dissolution medium. On the other hand, the SPE washing step allowed the elimination of SLS. The ursodeoxycholic acid method optimisation and validation were accomplished with no less than 80% in 30 min.

Conclusion: The developed analytical method was simple and adequate for the analysis of ursodeoxycholic acid suspension samples that met the USP specifications for dissolution test.

Keywords: ursodeoxycholic acid; suspension; dissolution; solid phase extraction, HPLC–UV

Introduction

Drug absorption from a solid and semisolid dosage form after oral administration depends on the release of the active pharmaceutical ingredient (API) from the product, the dissolution or solubilisation of the API under physiological conditions, and the permeation across the gastrointestinal membrane.[1]

Dissolution testing (DT) is an assay that provides a simple, cost-efficient and still rigorous test to evaluate drug release performance, especially for solid and semisolid pharmaceutical dosage form [2].

DT is used to monitor the release of the API from the oral pharmaceutical form before reaching the site of action and is also a regular quality control procedure applied in different stages of the production process such as formulation and stability study. In addition, DT is a powerful tool to estimate the *in vivo* drug performance.[3]

DT has also been applied to special dosage forms like pharmaceutical suspensions. In a previous work, two pharmaceutical suspensions of ursodeoxycholic acid (UDCA) have been developed.[4] UDCA is a naturally occurring bile acid, a steroid compound and hydroxyl derivative of 5β-cholan-24 oic acid[5] (Figure 1). It is a hydrophobic bile acid required for the oral treatment of hepatobiliary diseases, since it improves clinical symptoms, histological and biochemical parameters in pathologies with cholestasis.[6,7]

The biopharmaceutical classification system (BCS) groups APIs into four classes based on their solubility and intestinal permeability.[8] It establishes the regulatory requirements for the registration of a pharmaceutical product.[9] For APIs that are poorly soluble in water, as is the case with UDCA, the dissolution rate becomes the limiting factor for absorption.[10-11]

Analytical methods used for quantification of APIs from dissolution media require high sensitivity because of its expected low concentration in large volume media.

UDCA, like all bile acids, presents poor UV absorption. This fact is key to the selection of the analytical method. In this sense, HPLC methods coupled to different detectors applied to the quantification of UDCA in pharmaceutical samples have been reported, such as electrochemical and evaporative light scattering detector and refractive index detector.[12-14] Moreover, capillary electrophoretic methods have been reported for the analysis de UDCA in pharmaceutical samples.[15,16] However, those methodologies are not usually found in quality control laboratories.

Received: October 26, 2022. Editorial Acceptance: November 30, 2022
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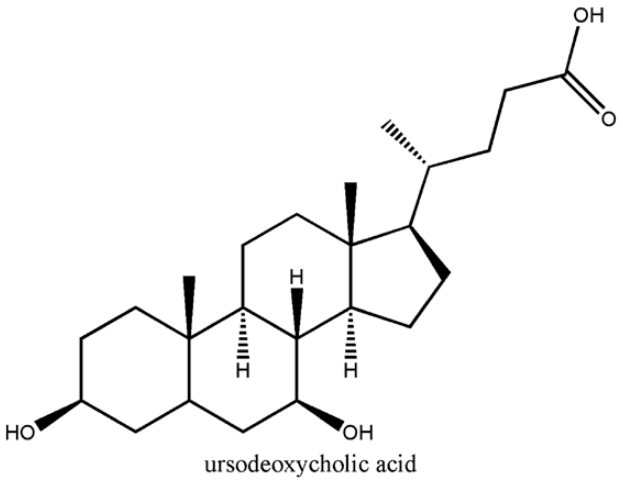

Figure 1 Chemical structure of ursodeoxycholic acid (UDCA).

HPLC–UV methods have been reported for the analysis of UDCA in different pharmaceutical samples (raw materials, tablets, suspension).[17,18] Recently, Khairy *et al.* have reported an HPLC–UV method for the simultaneous determination of UDCA and its epimer in tablets, using a conventional HPLC method and UHPLC method, in both cases at very low pH buffer in the mobile phase.[19] However, those methodologies have not been assayed in samples from dissolution media.

The USP dissolution test for UDCA solid dosage forms is a direct injection procedure using a refractive index detector. However, high amounts of surfactant are used in the dissolution medium (2% sodium lauryl sulphate [SLS] in 0.05 M phosphate buffer pH 8.4),[20] leading to problems in the chromatographic quantification of UDCA. Therefore, the samples obtained from the dissolution media must be clean up to remove SLS.

The aim of this work was to develop and validate for the first time an HPLC–UV method applied to a UDCA suspension for its quantification in dissolution medium with a previous sample preparation based on a solid phase extraction (SPE).

Materials and Methods

Chemical and reagents

UDCA standard and raw material were supplied by Sigma Aldrich (St. Louis, MO, USA) and Parafarm (Buenos Aires, Argentina) respectively. Xanthan gum, methylparaben (Nipagin), propylparaben (Nipasol), sodium saccharin, glycerine, and soy lecithin were provided by Magel S.A. (Buenos Aires, Argentina), dipotassium phosphate, monopotassium phosphate, phosphoric acid, ammonium acetate, methanol and acetonitrile (HPLC grade) were purchased from Merck (Darmstadt, Germany) and ultrapure water was obtained by an EASYpure RF equipment (Barnstead, Dudubuque, IA, USA). All solutions were filtered through a 0.45µm nylon membrane (Micron Separations Inc., Westboro, MA, USA) and degassed before use.

Equipment

- USP 40 apparatus 2. Not less than 80% of the labelled amount of UDCA must dissolve in 30 min. The paddle speed was set at 75 rpm.
- SPE cartridges: Strata C18-E (55 µm, 70A) 500 mg/3 ml, purchased from Phenomenex.
- HPLC–UV: Thermo Scientific SCM1000 with a quaternary pump, P4000 degasser, AS3000 autosampler, thermostatted column compartment and UV2000 detector (Waltham, Massachusetts, USA). Chromatograms were processed using ChromQuest 5.0 software.

Dissolution medium

Medium: 0.05 M pH 8.4 phosphate buffer, prepared by mixing 250 ml of 0.2 M monobasic potassium phosphate, 280 ml of 0.2 M potassium hydroxide, and 5 ml of 2 % SLS solution. Adjust with 0.2 M potassium hydroxide to a pH of 8.4 and dilute with water to 1000 ml (USP 40). [20]

Standard solution

A 5 mg/ml UDCA standard solution in methanol was prepared and a 1 ml dilution of this solution to 25 ml was made with dissolution medium followed by SPE.

Sample solution

Two different UDCA suspensions of 25 mg/ml (equivalent to 2.5%, w/v of UDCA) were prepared from raw material. Suspension A (SA) was prepared with minimal excipients, whereas Suspension B (SB) was prepared with different excipients and adjusted to pH 7 with monopotassium and dipotassium phosphate buffer. Reference suspension (SR) was prepared according to Santoveña *et al.*[11] Excipients for each formulation are listed in Table 1.

Experimental

UDCA analysis in suspensions

About 5 ml of each sample suspension was taken with a 5 ml disposable syringe and placed at the bottom of the dissolution vessel. All of the dissolution results were the average of three samples.

Dissolution test and SPE

The dissolution medium consisted of 900 ml of an aqueous solution of 0.05 M phosphate buffer (pH 8.4) with 2% SLS at according to USP 40.[20] After 30 min, 10 ml were taken with a 10 ml syringe. The samples were filtered and cleaned up with a SPE procedure. The cartridges were preconditioned with 2 ml of methanol followed by 2 ml of distilled water. About 5 ml of the sample were added, the unwanted retained compounds were washed with 2 ml of ultrapure water and then the UDCA extraction was carried out with 2 ml of methanol. The eluent was evaporated to dryness (rotary evaporator), and finally, the residue was dissolved in 5 ml with the mobile phase ACN:H_3PO_4 (pH 3.0, 0.15 mM) (48:52). The injection volume was 100 µl, the UDCA content was determined by HPLC with UV detection and analysed in triplicate, as explained by Boscolo *et al.*[17]

Chromatographic conditions

The chromatographic separation was carried out using a reverse phase Symmetry-C18 column (150 mm × 4.6 mm, id; particle size 5 µm), supplied by Waters (Milford, MA, USA). The mobile phase contained acetonitrile–phosphoric acid (pH

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Table 1 Suspension A (SA) and B (SB) composition for paediatric administration

	Functional category	Formulation (% w/v)		SR
		SA	SB	
Ursodeoxycholic acid, UDCA (g)	API	2.5	2.5	1.5
Xanthan gum (g)	Suspending agent	0.2	0.4	
Soy lechithin (g)	Suspending agent	–	0.1	
Methylcellulose (g)	Suspending agent	–	–	1
Glycerine (ml)	Humectant	5	5	20
Dipotassium phosphate (g)	Buffer	–	0.052	
Monopotassium phosphate (g)	Buffer	–	0.041	
Methylparaben (g)	Preservative	0.08	0.16	
Propylparaben (g)	Preservative	0.02	0.02	
Sodium saccharin (g)	Sweetening Agent	–	0.2	
Distilled water (ml)	Solvent	s.a.	s.a.	
Final pH		5.2	7.2	

s.a.= sufficient amount.
I (SB) = 0.02485 M (considering both phosphate salts and sodium saccharin).

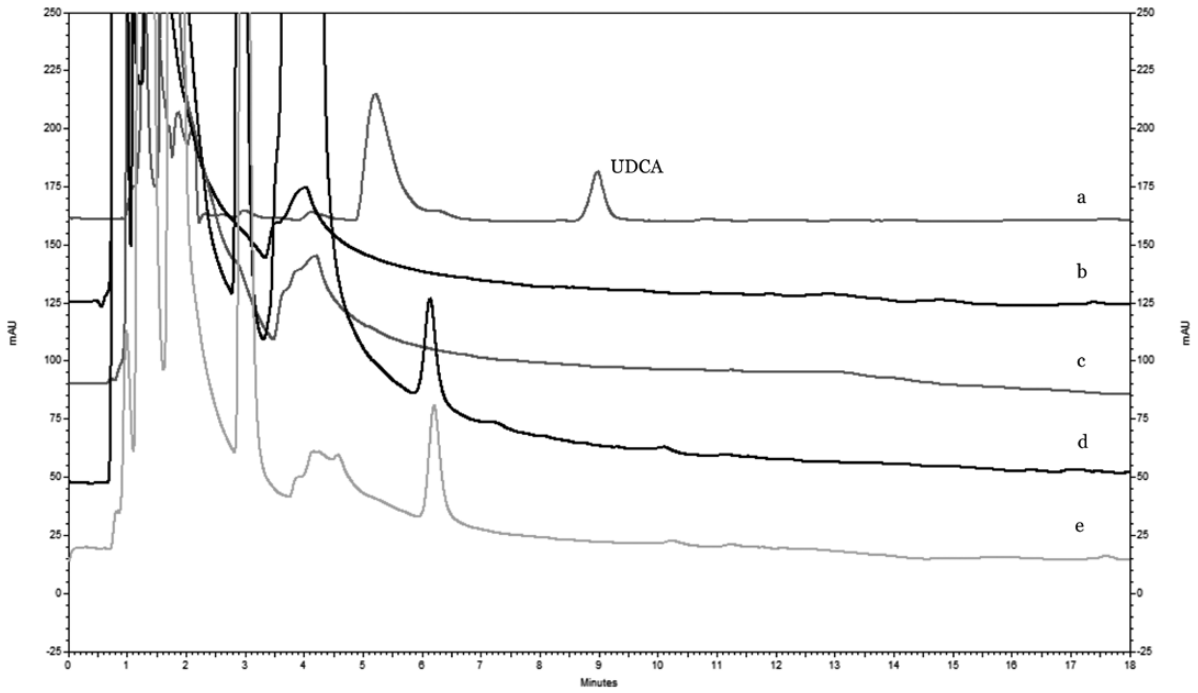

Figure 2 Chromatogram of (a) UDCA standard solution prepared with SPE procedure, (b) blank excipients, (c) UDCA standard solution, (d) suspension A, (e) suspension B. Solutions in chromatograms b, c, d and e were prepared without the SPE procedure.

3.0; 0.15 mM) (48:52). Isocratic separation was carried out with an injection volume of 100 µl, the flow rate was set at 1 ml/min and the column temperature was set at 40°C. The UV detection was carried out at 200 nm and all analysis was performed at 25°C. The retention time of the compound was 9 min.

Validation procedure

The validation of the HPLC–UV method was carried out according to the International Conference on Harmonization (ICH) guidelines for DT.[21] The following parameters were evaluated: specificity, linearity, LOD and LOQ, precision and accuracy. Specificity was evaluated by comparing the chromatograms of the blank of excipients of each pharmaceutical suspension with the UDCA standard solution. Linearity was performed at five UDCA concentration levels (100.0, 200.0, 250.0, 300.0 and 500.0 µg/ml), where each concentration was injected by triplicate. The LOD and LOQ were determined based on a signal-to-noise ratio of 3:1 and 10:1, respectively. Precision was evaluated intra-day (n = 6) and inter-day (n = 18) and RSD for peak area and retention times was determined. Accuracy was evaluated from recovery studies. Placebo samples containing all excipients were supplemented with UDCA at low (100.0 µg/ml), medium (250 µg/ml) and

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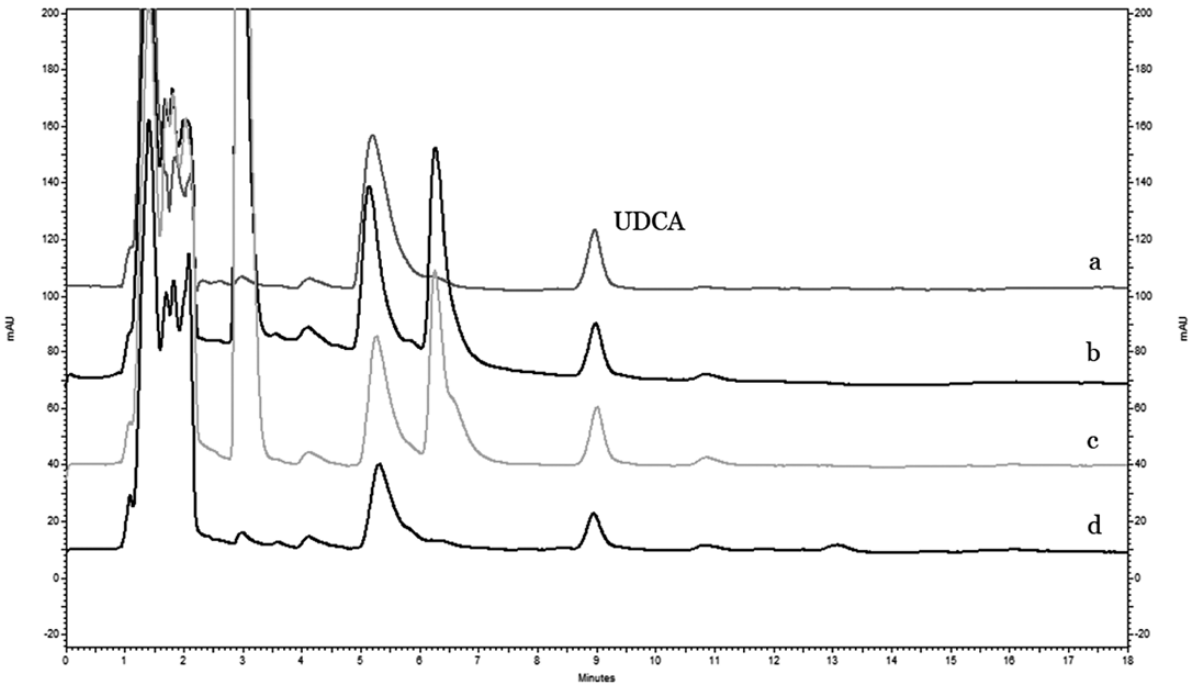

Figure 3 Chromatogram of (a) UDCA standard solution, (b) UDCA in suspension A, (c) UDCA in suspension B and (d) UDCA in suspension (SR). All solutions were prepared with the SPE procedure.

Table 2 Validation parameters for UDCA DT

Parameters	UDCA		
Linear and range (μg/ml)	100–600		
R^2	0.9909		
LOD (μg/ml)	0.33		
LOQ (μg/ml)	1.09		
Precision (RSD) of the method			
Intra-day (n = 6)			
Area	0.80		
Retention time	0.13		
Inter-day (n = 18)			
Area	1.86		
Retention time	0.44		
Accuracy and precision			
Spiked levels	Low	Medium	High
Suspension A	99.5 (0.5)	101.5 (0.6)	99.3 (0.2)
Suspension B	99.1 (0.9)	100.2 (0.7)	99.9 (0.8)

RSD values between brackets corresponding to n = 3.

high (500 μg/ml) concentration levels, and three replicates of each level were assayed.

Results and discussions

The SPE procedure has been optimised according to a previous work[22,23] regarding the analysis of bile acids in a biological matrix. In this sense, C18 was the best sorbent in terms of UDCA extraction efficiency and selectivity. Methanol was chosen for UDCA elution since it performed better than

Table 3 UDCA dissolution in SA, SB and SR

Suspension	% UDCA dissolved in 30 min[1]
SA	91.6 (0.6)
SB	97.2 (0.4)
SR	97.9 (1.4)

[1] Average of three samples; in brackets RSD values.

acetonitrile, isopropanol alone or in combination. In addition, the SPE procedure allowed the high recovery of UDCA from dissolution samples. The washing conditions of the SPE cartridges enabled the removal of SLS. Moreover, as seen in Figure 2, without SPE procedure, the UDCA peak is missing in both suspension samples. Therefore, the key to allow the detection of UDCA was the SPE procedure that was crucial to ensure the removal of SLS, which was an interferent in UDCA detection (Figure 3).

The optimised and validated analytical method was successfully applied to the analysis of UDCA from suspension samples. Validation parameters complied with international guidelines. Regarding specificity, no peaks were observed in the chromatogram of the blank of excipients, and the rest of the parameters are shown in Table 2. In both suspensions (A and B) and the reported reference suspension (SR), not less than 80% of UDCA dissolves in 30 min (Table 3). Therefore, both suspensions (A and B) meet the USP 40 specification for dissolution test.

Conclusion

A simple and effective HPLC method was developed and validated for the analysis of UDCA in dissolution samples, followed by an efficient and selective SPE extraction. The

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method complies with validation parameters in terms of accuracy, precision, specificity, linearity and range, and met the USP acceptance criteria and was successfully applied to the DT of UDCA suspensions.

Author's Contributions

Oriana Boscolo is the main researcher of the project and wrote the manuscript, Sabrina Flor performed the analytical experimental design, Cecilia Dobrecky revised the manuscript and supervised its content, Valeria Tripodi supervised the DT and Silvia Lucangioli is the research supervisor and is involved in all aspects of this work.

Funding

This work was supported by the University of Buenos Aires (UBACyT 20020170100405BA) and CONICET (PIP 11220170100717CO).

Conflict of Interest

The author(s) declare that there are no conflicts of interest.

Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article and can be found in the bibliographic reference by the DOI.

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