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Simultaneous quantification of simvastatin and excipients in liposomes using near infrared spectroscopy and chemometry

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ABSTRACT

This work describes the development and validation of a near infrared (NIR) spectroscopy method coupled with an appropriate multivariate calibration algorithm for the simultaneous quantification of encapsulated drug, simvastatin (SIM) and excipients, L-α-phosphatidylcholine (LPC) and cholesterol (CHO) in liposomes. The development of calibration models for each compound was based on a D-optimal experimental design consisting of 63 standard mixtures containing LPC, CHO and SIM in chloroform. For each compound, different spectral pretreatment methods were applied in association with selected spectral regions. Partial least-square regression (PLS) was performed using OPUS 6.5 software. Calibration set and cross-validation was carried out in order to select the best model to be used further. Straight line subtraction (SLS) was the best pre-treatment method for each compound, although the selected spectral regions were different. The method developed for each compound was validated in terms of linearity, trueness, precision and accuracy. Finally, the method has been successfully used for simultaneous quantification of SIM and excipients in liposomes. The encapsulation efficiency of SIM determined by this method was similar with that obtained by the use of reference HPLC method.

1. Introduction

There are many drugs that are currently investigated or even used as liposomal formulations, due to the ability of these carriers to increase their circulation time and therapeutic index. However, the pharmacokinetics and tissue distribution of liposomes depend on their physico-chemical properties such as size, surface charge, permeability, encapsulation volume, membrane composition and packing [1,2]. Thus, researchers are currently attempting to develop high-throughput methods for complete characterization of liposomes in terms of chemical composition (excipients and drug) and biorelevant physical properties.

Anticancer drugs are among the most studied candidates for encapsulation in carriers such as liposomes, because liposomes have the ability to increase the concentration of drug in the tumors. Statins, a class of therapeutic agents traditionally used to treat hypercholesterolemia and other cardiovascular diseases, have been recently shown to reduce cell viability and to induce apoptosis in various types of cancer cells, including colorectal [3], prostate [4], pancreatic [5], breast cancer [6], melanoma [7] and osteosarcoma [8,9]. Although the mechanism of anticancer activity of statins is not completely understood, it seems that the incubation of lipophilic statins with some cancer cell lines determine cellular death, depending on the type of statin, the administered dose, the exposure time and the type of cancer [10]. The lypophillic statins, simvastatin and lovastatin, are more active than the hydrophilic compounds [11]. Thus, in our research, simvastatin (SIM) was selected as the drug to be encapsulated in liposomes. Concerning the lipid composition of liposomes, L-α-phosphatidylcholine (LPC) and cholesterol (CHO) were chosen, as they represent the most commonly used excipients for the preparation of conventional liposomes.

Near infrared (NIR) spectroscopy has become an important tool of the pharmaceutical field for the characterization of raw materials, in-process characterization of products and characterization of dosage forms, due to its rapidity, simplicity, low-cost and non-destructive characteristic. This paper describes the development and validation of NIR-chemometric methods for simultaneous assay of LPC, CHO and SIM, and the use of these methods for characterization of SIM-loaded liposomes. The proposed methods have the advantage of giving information about chemical composition, yield of the preparation method and encapsulation efficiency, via a single analysis. It has been previously shown that NIR-chemometric methods are useful, reliable tools for chemical characterization of

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LPC–CHO liposomes [12]. The current work approaches the assay of both excipients (LPC and CHO) and encapsulated drug (SIM) in a single analysis. Moreover, NIR spectroscopy combined with chemometry was used for simultaneous assay of drugs or drug and excipients in various pharmaceutical formulations, such as tablets [13–15], mucoadhesive buccal gels [16] or powder blends for tableting [14,17].

2. Materials and methods

2.1. Materials

L-α-Phosphatidylcholine (egg lecithin, 80%) was from Lipoid GmbH (Germany), cholesterol (>92.5%, GC) from Sigma–Aldrich (Germany) and simvastatin (99.9%) was provided by Biocon Limited (India). All the other solvents and reagents (chloroform, ethanol, phosphates) were of analytic grade purity, commercially available.

2.2. Samples

2.2.1. Preparation of calibration samples

A training calibration set of 63 standard mixtures containing LPC, CHO and SIM in chloroform was prepared according to a D-optimal experimental design with 3 variables and 5 levels, developed in Modde 9.0 Software (Umetrics, Sweden). For this purpose, stock solutions of LPC (40 mg/ml), CHO (4 mg/ml) and SIM CHO (3.2 mg/ml) were separately prepared by dissolving 1000 mg LPC, 100 mg CHO and 80 mg SIM in 25 ml of chloroform, respectively. The stock solutions were subsequently diluted with appropriate volume of chloroform, in order to obtain 5 standard solutions of LPC (4, 5.5, 7, 8.5 and 10 mg/ml), CHO (0.4, 0.55, 0.7, 0.85 and 1.0 mg/ml) and SIM (0.04, 0.23, 0.42, 0.61 and 0.8 mg/ml). Finally, stock solutions of the three components were mixed in appropriate volumes and diluted with chloroform to give reference mixtures with concentration values within the range of 4–10 mg/ml for LPC, 0.4–1 mg/ml for CHO and 0.04–0.8 mg/ml for SIM.

2.2.2. Preparation of validation samples

The NIR method was validated in order to demonstrate that this analytical tool is suitable for its intended use. For validation purpose, 3 independent series (corresponding to 3 different days) were prepared at 3 concentration levels for LPC (5.5, 7 and 8.5 mg/ml), CHO (0.55, 0.7 and 0.85 mg/ml) and SIM (0.23, 0.42 and 0.61 mg/ml). For each concentration level 4 independent mixtures were prepared and analyzed (12 samples/day). The validation set was prepared using stock solutions prepared in the same manner as those used for the preparation of calibration samples.

2.2.3. Preparation of liposomes

The liposomes were prepared using the "film method". Briefly, LPC (60 μM/ml), CHO (12 μM/ml) and SIM (12 μM/ml) were dissolved in 5 ml ethanol in a round-bottomed flask. After complete dissolution, the solvent was evaporated under reduced pressure at 50 °C in a rotary evaporator, leading to the formation of a thin film on the surface of the flask. In order to completely remove the residual solvent, the film was maintained under a nitrogen gas flow for 1 h. Then the film of lipids was hydrated with 5 ml phosphate buffered saline (PBS, pH = 7.8) for 15 min at 40 °C. The liposomes were separated by centrifugation (15 min, 10,000 × *g*) and were finally diluted to 5 ml with the hydration buffer and stored at 4 °C until analysis.

2.3. Calibration and validation protocol

Calibration and validation samples were prepared and analyzed in 3 different days (3 series), using freshly prepared stock solutions of LPC, CHO and SIM. The 100% LPC content has been considered 7 mg/ml, close to the usual targeted concentration of LPC in liposomes samples. The standard mixtures in the calibration set were prepared in a concentration range of 60–140% of usual LPC content (60, 80, 100, 120 and 140%), according to D-optimal experimental design. The validation has been performed on independent samples containing 80, 100 and 120% of usual LPC content, in 4 replicates for each concentration level. Similarly, the concentration of 0.7 mg/ml has been considered as 100% CHO content and the standard mixtures in the calibration set were prepared in a concentration range of 60–140% of usual CHO content, according to D-optimal experimental design. The validation set consisted in independent samples at 3 concentration levels in the range 80–120%, each concentration (80, 100 and 120%) in 4 replicates. Finally, the concentration of 0.42 mg/ml has been considered as 100% SIM content. The calibration set for SIM was prepared in the range 10–190% around this concentration, according to D-optimal experimental design. The validation set for SIM contained sample at the concentration levels

Table 1
Composition of calibration set, according with a D-optimal experimental design.

Exp. name	X_1 (μg/ml)	X_2 (μg/ml)	X_3 (μg/ml)	Exp. name	X_1 (μg/ml)	X_2 (μg/ml)	X_3 (μg/ml)	Exp. Name	X_1 (μg/ml)	X_2 (μg/ml)	X_3 (μg/ml)
N1	4000	400	40	N22	7000	850	230	N43	10,000	550	610
N2	7000	400	40	N23	10,000	850	230	N44	5500	700	610
N3	10,000	400	40	N24	5500	1000	230	N45	8500	700	610
N4	5500	550	40	N25	8500	1000	230	N46	4000	850	610
N5	8500	550	40	N26	4000	400	420	N47	7000	850	610
N6	4000	700	40	N27	7000	400	420	N48	10,000	850	610
N7	7000	700	40	N28	10,000	400	420	N49	5500	1000	610
N8	10,000	700	40	N29	5500	550	420	N50	8500	1000	610
N9	5500	850	40	N30	8500	550	420	N51	4000	400	800
N10	8500	850	40	N31	4000	700	420	N52	7000	400	800
N11	4000	1000	40	N32	7000	700	420	N53	10,000	400	800
N12	7000	1000	40	N33	10,000	700	420	N54	5500	550	800
N13	10,000	1000	40	N34	5500	850	420	N55	8500	550	800
N14	5500	400	230	N35	8500	850	420	N56	4000	700	800
N15	8500	400	230	N36	4000	1000	420	N57	7000	700	800
N16	4000	550	230	N37	7000	1000	420	N58	10,000	700	800
N17	7000	550	230	N38	10,000	1000	420	N59	5500	850	800
N18	10,000	550	230	N39	5500	400	610	N60	8500	850	800
N19	5500	700	230	N40	8500	400	610	N61	4000	1000	800
N20	8500	700	230	N41	4000	550	610	N62	7000	1000	800
N21	4000	850	230	N42	7000	550	610	N63	10,000	1000	800

X_1, lecithin; X_2, cholesterol and X_3, simvastatin.