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Development of a HILIC method for the determination of 5-fluorouracil from nano drug delivery systems and rat skin extracts

Gulin Amasya[a,*], Mehmet Gumustas[b,c,**], Ulya Badilli[a], Sibel. A. Ozkan[d], Nilufer Tarimci[a]

[a] Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Ankara, Turkey
[b] Ankara University, Institute of Forensic Sciences, Department of Forensic Toxicology, Ankara, Turkey
[c] Hitit University Faculty of Arts & Sciences, Department of Analytical Chemistry, Corum, Turkey
[d] Ankara University, Faculty of Pharmacy, Department of Analytical Chemistry, Ankara, Turkey

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ABSTRACT

This is the first report in literature using hydrophilic interaction liquid chromatography (HILIC) in combination with diode array detector (DAD) for stability indicating determination of 5-Fluorouracil (5-FU) from its bulk form, pharmaceutical preparations, developed solid lipid nanoparticle (SLN) and nano structured lipid carrier (NLC) drug delivery systems as well as the rat skin extracts. The separation was performed at 45 °C, on Sequant Zic HILIC (250 mm × 4.60 mm ID, 5 μm, 200 Aº), peek HPLC column. Mobile phase is consisting of a mixture of acetonitrile: buffer containing 5 mM ammonium acetate (95:5; v/v). The pH of the mobile phase was adjusted to 7.0 using 1 M NaOH. The analysis was carried out at 0.75 mL min^{-1} flow rate with a detection wavelength of 265 nm and the injection volume was arranged as 10 μL. The developed method was fully validated in accordance with the International Council on Harmonization (ICH) Guidelines. Specificity of this method was demonstrated by forced degradation studies. As a result of calibration studies, the calibration curve was found linear in the concentration range of 1–250 μg mL^{-1} (R^2 = 0.999). The precision of this technique calculated within the frame of intra-day and inter-day based on a percentage of relative standard deviation (RSD%) values (<2%). The limits of detection and quantification were 11 and 37 ng mL^{-1} respectively. On the other hand, 5-FU loaded SLN and NLC formulations with average particle size of 370 nm were also developed and compared in order to increase the permeation of drug into the rat skin. Ex-vivo Penetration/Permeation Studies indicated that higher dermal accumulation of 5-FU was obtained with NLC formulation. As a conclusion, the present work expressed the optimization and the validation of a selective, simple, precise and accurate fully validated HILIC method with sufficient sensitivity for the estimation of 5-FU in raw materials, marketed formulation and rat skin extract after applying both of the commercial product and newly developed nanoparticulate drug delivery systems on to the rat skins with high percentage recoveries.

1. Introduction

5-FU (5-fluoropyrimidine-2,4(1H,3H)-dione) is a topically used anticancer agent for the treatment of actinic (solar) keratosis and basal cell carcinomas of the skin [1]. It is a pyrimidine antagonist and acts as an inhibitor of the DNA or RNA synthesis [2]. Marketed products containing 5-FU (0.5–5%) in dosage forms of creams and solutions are currently used for skin cancer treatment [3]. However, the hydrophilic nature of 5-FU limits the penetration of the drug into the skin layers [4,5].

Both SLNs and NLCs receive considerable interest for application of drugs through the dermal route. SLNs are colloidal drug delivery systems which are produced with solid lipids such as fatty acids (e.g. stearic acid), triglycerides (e.g. tristearin), partial glycerides (e.g. glyceryl behenate) and waxes (e.g. cetly palmitate) or mixtures of them. These lipids are classified in the GRAS (Generally Recognized as Safe) category [6–8]. SLNs offer several advantages such as high physical stability, producibility without organic solvents, feasibility for manufacturing on an industrial scale, enhancement in stability of active substances, controlled drug release and drug

* Corresponding author.
** Corresponding author at: Ankara University, Institute of Forensic Sciences, Department of Forensic Toxicology, 06590, Ankara, Turkey.
E-mail addresses: gamasya@pharmacy.ankara.edu.tr (G. Amasya), mgumustas@ankara.edu.tr (M. Gumustas).

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targeting [9–11]. However, relatively low drug loading in SLNs can be obtained especially for the hydrophilic active substances. On the other hand, drug leakage from carrier system can occur because of the polymorphic transitions during the storage period [12,13]. In order to eliminate these disadvantages, NLCs were developed in the early 2000s. NLCs, the second-generation lipid nanoparticles, are manufactured with the mixture of liquid lipids and solid lipids. The lipid matrix of NLCs has numerous imperfection zones and this structure allows a large space for incorporation of the drug molecules [14–16].

The bibliography concerning those new systems for delivery of active pharmaceutical ingredients is huge and is in constant evolution. It deals with preparation, characterization, and determination of 5-FU loaded delivery systems and shows promising results. Based on a literature survey, several reversed phase HPLC methods were reported for the determination of 5-FU in pharmaceutical dosage forms [15,17–21]. Moreover, LC–MS/MS was used for the determination of 5-FU from biological matrices with using also in reversed phase mode [22]. However, the present procedure is presented also the stability indicating degradation behaviour of the 5-FU like in the literature [15,17–21]. These reports were selective enough to resolve the active ingredients from their potential impurities and forced-degradation products. However, this is the first report in the literature using a HILIC in combination with diode array detector (DAD) for the stability indicating determination of 5-FU from its bulk form, pharmaceutical preparations, developed SLN and NLC drug delivery systems as well as the rat skin extracts.

The HILIC technique proposed by Alpert in 1990 has been applied for analysis of many hydrophilic compounds. As a hydrophilic stationary phase, amide, diol, polyol, bare silica, ion exchange and zwitterion type stationary phases have been used along with high percentage of organic solvent as mobile phase for HILIC. It is somehow a defined combination of normal phase and reversed phase as well as the ion chromatography [23]. Therefore, it is an interesting alternative for the analysis of polar substances and can be defined as a separation method that combines stationary phases usually used in normal phase and mobile phases used in reversed phase separations. The mechanism on silica surface can be explained by the combination of the mechanisms following, polar analyte partitions into and out of adsorbed water layer and also it can undergo cation exchange with charged silanol groups. Apart from retention of highly polar analytes, this technique has more benefits like complementary selectivity to the reversed phase, enhanced sensitivity in mass spectrometry [23,24].

This study brings an innovation for the selective and reliable determination of 5-FU. Ex-vivo percutaneous permeation/penetration studies of 5-FU loaded SLN and NLC were also performed and dermal behaviour of the nanoparticles was evaluated. The skin transport of 5-FU which has low percutaneous permeation and poor dermal bioavailability was enhanced by NLC compared to SLN from the analytical point of view. The goal of the present work is to optimize and develop a selective, simple, precise and accurate fully validated HILIC method with sufficient sensitivity for the estimation of 5-FU in raw materials, marketed formulation, and rat skin extract after applying both of the commercial product and newly developed nanoparticulate drug delivery systems.

2. Materials and methods

2.1. Instrumentation

The Agilent 1100 series LC system (Wilmington, DE, USA) with DAD was used for the development of the HILIC method and validation studies. The analysis was performed at 45 °C using Sequant Zic HILIC (250 mm × 4.60 mm ID, 5 μm, 200 A°) (Merck KGaA, Darmstadt, Germany), column as the stationary phase. Chromatographic grade water was obtained through a Milli-Q® system (Millipore, Milford, MA, USA) and was used to prepare all necessary solutions. Primary was prepared using high shear homogenizer at 20.500 rpm (Ultra-Turrax T25, IKA Labortechnik, Staufen im Breisgau, Germany). After that water/oil/water (W/O/W) double emulsion was obtained using probe sonicator at 70W of energy output (Bandelin Sonoplus HD 2070, Bandelin Elec., Berlin, Germany). Lipid nanoparticles were obtained after the removing of organic solvent. The nanoparticle suspension was centrifuged at 57.000 × *g* (Sigma Laboratory Centrifuge 3KS30, St. Louis, Missouri, USA). For the lyophilization procedure, Christ Gamma 2–16 LSC lyophilizator (Martin Christ Gef., Osterode am Harz, Germany) was used. A dynamic light-scattering analyser (Nano ZS, Malvern Inst., Malvern, Worcestershire UK) was used to measure the mean particle size and polydispersity index of the lipid nanoparticles. The zeta potential was determined from the electrophoretic mobility on Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). The thermal properties and crystallinity of pure 5-FU, pure solid lipid, SLN and NLC formulations were examined by differential scanning calorimeter (Shimadzu DSC 60, Shimadzu, Kyoto, Japan).

2.2. Chemicals and reagents

All chemicals and solvents were analytical reagent grade. 5-Fluorouracil was kindly provided by the Deva Drug Company (Istanbul,Turkey). Compritol 888 ATO was kindly requested by Gattefossé (Saint-Priest, France). Transcutol® (Diethylene glycol monoethyl ether) and Tween 80® were purchased form Sigma-Aldrich (Munich, Germany). Chromatography grade acetonitrile, analytical grade phosphoric acid (85%), sodium hydroxide, potassium bromide, toluene, were purchased from Sigma-Aldrich (Munich, Germany).

2.3. Conditions for the chromatographic system

The separation was performed at 45 °C, on a Sequant Zic HILIC (250 mm × 4.60 mm ID, 5 μm, 200 A°), Peek HPLC column. Each working day injection was carried out after pre-conditioning of the column at the optimized temperature for approximately 20 min.Mobile phase consisting of a mixture of acetonitrile: buffer containing 5 mM ammonium acetate (95:5; v/v) was used for the analysis of active pharmaceutical ingredient. The pH of the mobile phase was adjusted to 7.0 using 1 M NaOH. An ultrasonic bath was used for degassing of the mobile phases then these solutions were filtered under a vacuum by using a 0.45 μm filter. The analysis was carried out at 0.75 mL min^{-1} flow rate with a detection wavelength of 265 nm and the injection volume was arranged as 10 μL.

2.4. Preparation of standard solutions

A stock solution of 5-FU (0.5 mg mL^{-1}) was prepared by dissolving 5-FU in acetonitrile. Calibration was constructed by diluting the stock solutions with acetonitrile in the range of 1–250 μg mL^{-1}. The calibration curve was also constructed by plotting the peak area of 5-FU against the concentration. All calibration levels were analyzed three times in order to show the precision of the calibration standard solutions. For intraday and inter-day repeatability studies, three levels of concentration that are from the linearity-range selected and prepared each day for three consecutive days. All these solutions were kept at 4 °C in a refrigerator.