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Stir bar sorptive extraction of diclofenac from liquid formulations: A proof of concept study

Prashant Laxman Kole*, Jeff Millership, James C. McElnay

PKPD Core Facility, School of Pharmacy, Queens University Belfast, 97 Lisburn Road, Belfast BT9 7BL, United Kingdom

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ABSTRACT

A new stir bar sorptive extraction (SBSE) technique coupled with HPLC-UV method for quantification of diclofenac in pharmaceutical formulations has been developed and validated as a proof of concept study. Commercially available polydimethylsiloxane stir bars (Twister™) were used for method development and SBSE extraction (pH, phase ratio, stirring speed, temperature, ionic strength and time) and liquid desorption (solvents, desorption method, stirring time etc) procedures were optimised. The method was validated as per ICH guidelines and was successfully applied for the estimation of diclofenac from three liquid formulations viz. Voltarol® Optha single dose eye drops, Voltarol® Ophtha multidose eye drops and Voltarol® ampoules. The developed method was found to be linear ($r = 0.9999$) over 100–2000 ng/ml concentration range with acceptable accuracy and precision (tested over three QC concentrations). The SBSE extraction recovery of the diclofenac was found to be 70% and the LOD and LOQ of the validated method were found to be 16.06 and 48.68 ng/ml, respectively. Furthermore, a forced degradation study of a diclofenac formulation leading to the formation of structurally similar cyclic impurity (indolinone) was carried out. The developed extraction method showed comparable results to that of the reference method, i.e. method was capable of selectively extracting the indolinone and diclofenac from the liquid matrix. Data on inter and intra stir bar accuracy and precision further confirmed robustness of the method, supporting the multiple re-use of the stir bars.

1. Introduction

Pharmaceutical formulations are mixtures of a drug, i.e. the intended therapeutic entity, and various excipients. At any given time, the formulation might also contain impurities from the raw material (drug/excipients) and degradation products resulting from various storage conditions and/or drug–excipient interactions. In recent years, there have been considerable developments in analytical techniques resulting in increased selectivity and sensitivity of analytical methods, however, even with various highly efficient analytical instruments, sample preparation procedures are usually necessary to extract and isolate the analytes of interest from complex matrices as most analytical instruments cannot handle the matrix directly [1].

Conventionally, solid–liquid extraction (SLE) [2–4] and/or liquid–liquid extraction (LLE) [5–7] are used for sample preparation purposes with drug formulations, however, in such a situation, if selectivity is in question (interference from impurities/degradation products etc at the retention time of analyte of interest), then further sample clean up is required. In recent years, Solid phase extraction (SPE) has increasingly been used to extract and estimate drugs [8], excipients [9] or degradation products [10] in pharmaceutical formulations especially, when a method needs to be stability indicating or the extraction involves a complex formulation matrix such as a cream [8]. Despite obvious advantages of SPE, one of the major factors associated with this technique is its cost along with other problems such as clogging/plugging of cartridges, channelling etc [11].

More recently, there have been many developments in the field of sample preparation techniques. In 1990, Pawliszyn and Arthur developed a new sample-preparation technique using a fused-silica fiber coated on the outside with an appropriate stationary phase; this is termed solid phase micro-extraction (SPME) [12]. In contrast to conventional SPE with packed-bed cartridges, the SPME syringe assembly design allows the combination of all the steps of sample preparation into one step and thus reduces sample preparation time, the use of organic solvents and disposal costs. The foremost advantage of the technique is improved detection limits [13]. A development of SPME, stir bar sorptive extraction (SBSE) was introduced as a novel sample preparation technique in 1999 [14]. SBSE is a sorptive and (in general) solventless extraction technique based on the same principles as SPME, but, instead of a polymer-

* Corresponding author. Tel.: +44 28 90972361; fax: +44 28 90247794.
E-mail address: prashantkole@gmail.com (P.L. Kole).

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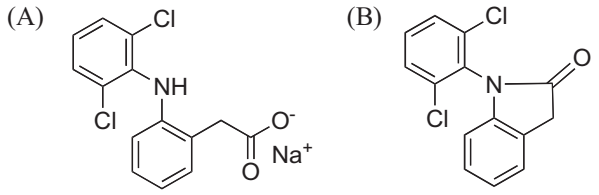

Fig. 1. Chemical structure of diclofenac (A), indolinone derivative of diclofenac (B).

coated fiber, a large amount of the extracting phase is coated on to a stir-bar. Extraction of an analyte from the aqueous phase sample into an extraction medium (e.g. polydimethylsiloxane (PDMS)) is controlled by the partitioning coefficient of the analyte between the silicone phase and the aqueous phase (K_{PDMS}/w) [15]. This partitioning coefficient is well correlated with octanol–water distribution coefficients ($K_{o/w}$) of the drugs. Due to the similarity of K_{PDMS}/w to $K_{o/w}$, chemists can predict extraction efficiencies (SBSE can be efficiently used for hydrophobic compounds with $\log K_{o/w} \geq 2$; and, a high enrichment factor could be obtained for analytes with $\log K_{o/w} > 5$) [15,16]. SBSE based methods have been traditionally used for estimation of various organic analytes such as polycyclic aromatic hydrocarbons (PAH) from aqueous samples/drinking water [17,18], pesticides [19], trace residues and contaminants in foods [20] etc. Most recently, SBSE has been used for extraction of drugs from various biological matrices such as urine [21,22], plasma [23–25] and tissues [26]. Application of SBSE to the extraction and estimation of drugs/impurities in pharmaceutical formulations has not been published in the literature.

The method presented in this paper is a proof of concept study for investigation of the applicability of the SBSE technique in the determination of a drug in liquid formulations. Diclofenac sodium was chosen as model drug. Diclofenac [2-[(2,6-dichlorophenyl)-amino-phenyl]acetic acid] (Fig. 1) is a synthetic non steroidal anti-inflammatory drug (NSAID) usually available as the sodium or potassium salt [27], and is widely used as an analgesic, anti-inflammatory and anti-arthritic agent [28]. Dosage forms for diclofenac (DIC) include tablets, capsules, gels, aerosols, ointments, suppositories, parenteral injections, eye drops (single and multiple use) containing varying amounts of DIC. For the present study estimation of DIC in liquid formulations i.e. eye drops (single and multiple uses) and injection was chosen. A literature survey for analytical methods available for estimation of DIC in formulations revealed many methods utilising a variety of analytical techniques such as UV spectroscopy [29], HPLC coupled with UV detection [29–31], HPLC coupled with fluorescence detection [32], HPLC coupled with mass spectrometry [33,34], capillary electrophoresis [35], potentiometric [36], gravimetric [37], densitometric [38], diffuse reflectance photometry [27], FT-Raman spectroscopy [39]. Further evaluation of the literature revealed sample preparation techniques such as hollow fiber-based liquid phase microextraction (HF-LPME) coupled with HPLC and diode array (DAD)–fluorescence (FLD) detectors (in series) for extraction of DIC from an aqueous matrix [40]. A literature survey for SBSE and DIC estimation revealed one method for the estimation of DIC in environmental water matrices [41].

The objective of the present study was to develop and validate a SBSE method for estimation of DIC and its application for determination of DIC in eye drops and injection formulations. Further, it was also intended to apply the developed method for determination of DIC from stressed injection formulations (autoclave and dry heat) and to assess the ability of the methodology to detect and quantify the structurally similar impurity/degradation product 1-(2,6-dichlorophenyl)-indolin-2-one (the indolinone derivative of DIC).

2. Materials and methods

2.1. Chemicals and materials

Diclofenac sodium was purchased from Sigma–Aldrich Ltd (Poole, UK). HPLC grade methanol and acetonitrile were supplied by Fisher Scientific (Loughborough, UK). HPLC grade water was obtained using a Millipore Direct-Q™ 5 Water System (Millipore, Watford, UK). Analytical grade sodium chloride and di-sodium-hydrogen phosphate were purchased from BDH (Poole, UK). Indolinone derivative of the diclofenac was synthesised, purified in-house and assayed for its content in the laboratory. All other reagents were of analytical grade except where otherwise stated. The diclofenac liquid formulations viz. Voltarol® Optha single dose drops (0.1% (w/v)), Voltarol® Ophtha multidose eye drops (0.1% (w/v)) and Voltarol® ampoules (75 mg/3 ml) were obtained from AAH Pharmaceuticals Ltd., Belfast, UK.

2.2. SBSE accessories

Four commercially available stir bars (Twister™) varying in length and thickness of polydimethylsiloxane (PDMS) (0.5 mm thickness and 10 mm length (PDMS volume ~24 μl), 1 mm thickness and 10 mm length (PDMS volume ~63 μl), 0.5 mm thickness and 20 mm length (PDMS volume ~47 μl) and 1 mm thickness and 20 mm length (PDMS volume ~126 μl)) were purchased from Gerstel (Gerstel GmbH, Mulheim Ruhr, Germany). The stir bars were pre-conditioned by sonication in a mixture of dichloromethane and methanol (1:1, v/v) for 10 min and dried with lint-free tissue. The dried stir bars were heated at 200 °C for 15 min before being used for extraction. A 15 position magnetic stirrer (0–1200 RPM) with integrated temperature control plate (IKA® multi position hotplate stirrer RT 15) was purchased from VWR International, UK.

2.3. Preparation of stock solutions, calibration standards and quality control (QC) samples

2.3.1. Stock solutions

A primary stock (PS) solution of DIC was prepared in methanol at 1 mg/ml (1000 μg/ml). The PS solution was diluted with methanol to give a secondary stock (SS) solution of 100 μg/ml. Working standards (WS) at 4, 10, 20, 30, 40, 60 and 80 μg/ml were prepared in methanol from the SS solution. Analytical standards (AS) at 100, 250, 500, 750, 1000, 1500, 2000 ng/ml were prepared in mobile phase by using respective working standards. All the stock solutions PS, SS and WS were stored at refrigerated condition (4 °C).

2.3.2. Aqueous calibration standards (ACS) and QC standards

An aqueous phase (AP) containing 15% (w/v) of sodium chloride was prepared in bulk. The pH of this AP was adjusted to 2.5 using hydrochloric acid. The 5 ml ACS standards were prepared by spiking AP with 25 μl of appropriate WS (so as to give 100, 250, 500, 750, 1000, 1500 and 2000 ng/5 ml). Similarly, three QC standards i.e. 100 ng/5 ml (LQC), 750 ng/5 ml (MQC) and 2000 ng/5 ml (HQC) were also prepared and further used in validation of the method. ACS and QC samples were spiked taking consideration of the final reconstitution volume of 1 ml which will yield 100, 250, 500, 750, 1000, 1500 and 2000 ng/ml concentrations. The total concentration of organic solvent in 5 ml ACS was not more than 0.5% (v/v).

2.4. Chromatographic system

The chromatography was carried out using the Waters® Alliance HPLC system (Waters, Ireland) which consisted of a Waters® 2695 separations module and a Waters® 2487 dual