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Rifampicin determination in plasma by stir bar-sorptive extraction and liquid chromatography

Marina Salviato Balbão[a], Carlo Bertucci[b], Mateus Machado Bergamaschi[a], Regina Helena Costa Queiroz[a,*], Wilson Roberto Malfará[a], Sônia Aparecida Carvalho Dreossi[a], Lidervan de Paula Mello[c], Maria Eugênia Costa Queiroz[c]

[a] Department of Clinical, Toxicologic and Bromatologic Analyses, Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Brazil
[b] Department of Pharmaceutical Sciences, University of Bologna, Italy
[c] Departamento de Química, Faculdade de Filosofia Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Brazil

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ABSTRACT

A sensitive and reproducible stir bar-sorptive extraction and high performance liquid chromatography-UV detection (SBSE/HPLC-UV) method for therapeutic drug monitoring of rifampicin in plasma samples is described and compared with a liquid:liquid extraction (LLE/HPLC-UV) method. This miniaturized method can result in faster analysis, higher sample throughput, lower solvent consumption and less workload per sample while maintaining or even improving sensitivity. Important factors in the optimization of SBSE efficiency such as pH, temperature, extraction time and desorption conditions (solvents, mode magnetic stir, mode ultrasonic stir, time and number of steps) were optimized recoveries ranging from 75 to 80%. Separation was obtained using a reverse phase C_8 column with UV detection (254 nm). The mobile phase consisted of methanol:0.25 N sodium acetate buffer, pH 5.0 (58:42, v/v). The SBSE/HPLC-UV method was linear over a working range of 0.125–50.0 $\mu g\,mL^{-1}$. The intra-assay and inter-assay precision and accuracy were studied at three concentrations (1.25, 6.25 and 25.0 $\mu g\,mL^{-1}$). The intra-assay coefficients of variation (CVs) for all compounds were less than 10% and all inter-CVs were less than 10%. Limits of quantification were 0.125 $\mu g\,mL^{-1}$. Stability studies showed rifampicin was stable in plasma for 12 h after thawing; the samples were also stable for 24 h after preparation. Based on the figures of merit results, the SBSE/HPLC-UV proved to be adequate to the rifampicin analyses from therapeutic to toxic levels. This method was successfully applied to the analysis of real samples and was as effective as the LLE/HPLC-UV method.

1. Introduction

Rifampicin (RIF) is categorized as one of the first line antituberculous agents. The ability to kill *Mycobacterium tuberculosis* is related to the concentration of drug to which the bacterium is exposed. Incomplete treatment of tuberculosis (TB), is common and the development of drug resistance [1] may usually be attributed to non-compliance with the therapeutic regime or an interrupted supply of drugs. Therapeutic drug monitoring (TDM) [2] may provide a means of determining compliance, particularly in remote areas of developing countries. Currently, plasma levels of RIF (Fig. 1) are not monitored routinely in TB patients but it is clear that this would be advantageous if a simple and effective quantitative test was available. A number of HPLC-based assays for rifampicin have been described [2–14]. Analytical methods generally require an extraction and enrichment before an analyst can perform the chromatographic separation and detection of organic compounds in aqueous matrices. Solid-phase microextraction (SPME) was successfully applied to analyze drugs in biological fluids by chromatography techniques. The principles and applications of sorptive extraction for sample preparation have been reviewed by Kawaguchi et al. [15], David and Sandra [16] and Lanças et al. [17]. A glass stir bar is coated with a potentially thick bonded absorbent layer (polydimethylsiloxane, PDMS) to give a large surface area of stationary phase, leading to a higher phase ratio and hence a better recovery and sample capacity. Transfer of the analyte from the bar is achieved either by elution with a LC solvent or GC thermal desorption. Those techniques include SPME and stir bar-sorptive extraction (SBSE). Sorptive extraction has proven to be an interesting and environmentally friendly alternative to liquid extraction. In sorptive extraction, the analytes are extracted

* Corresponding author at: Departamento de Análises Clínicas, Toxicológicas and Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Avenida do Café s/n, Monte Alegre, CEP 14040-903 Ribeirão Preto, São Paulo, Brazil.
E-mail address: rqueiroz@fcfrp.usp.br (R.H.C. Queiroz).

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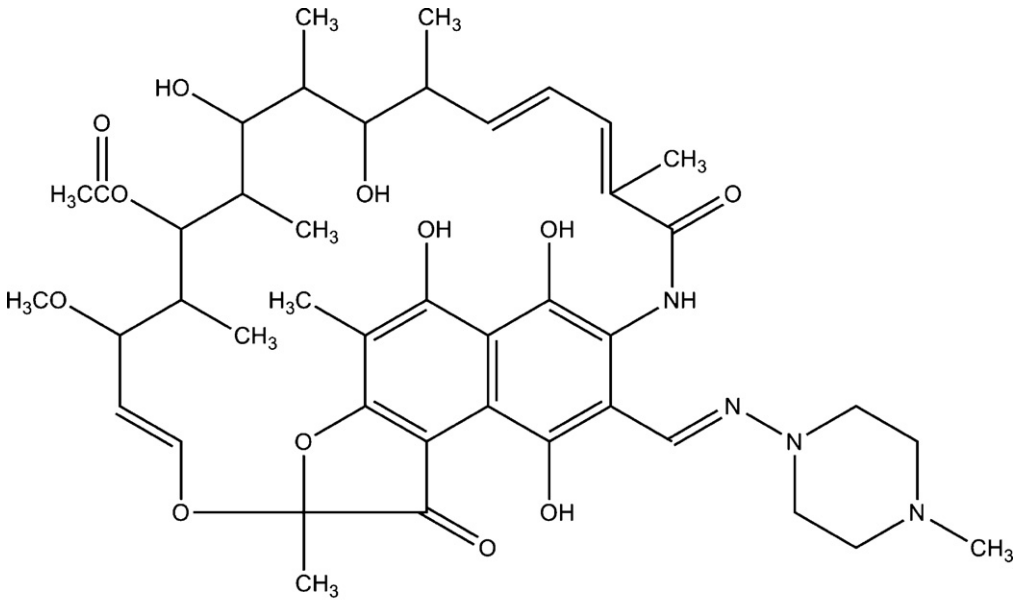

Fig. 1. Chemical structure of the rifampicin and sulindac (IS).

from the matrix (mostly aqueous) into a non-miscible liquid phase. In contrast to extraction with adsorbents in which the analytes are bound to the active sites on a surface, not only the surface area but also the total amount of the extraction phase is important in sorptive extraction. The main difference between SPME and SBSE is the much larger volume of PDMS used in SBSE, which results in higher recoveries and higher sample capacity. Kawaguchi et al. [15] also reported that the major advantage of SBSE is the higher concentration factors that can be achieved when theoretical recovery reaches 100% for solutes with $K_{O/W}$ values lower than 500 ($\log P$ greater than 2.7). RIF theoretical recoveries can be calculated for a given sample volume, selected stir bar dimensions, and KowWin around 4.8.

Recently, various methods involving SBSE were developed in order to further facilitate analysis and improve sensitivity. Novel methods that involve SBSE with in situ derivatization, SBSE with in situ de-conjugation, thermal desorption (TD) in the multi-shot mode and TD with in tube derivatization method. Those methods were applied successfully to biological samples [18–30]. The analytical methods described in the literature to analyze RIF in biological fluids usually adopt conventional sample pre-treatment techniques that are laborious, time-consuming and require large amounts of organic solvents.

The purpose of the present report is to quantify plasma RIF concentrations in tuberculosis patients using SBSE and compared with a conventional sample pre-treatment technique based on liquid:liquid extraction (LLE), also developed and validated in our laboratory, followed by HPLC-UV.

2. Experimental

2.1. Standards and chemicals

RIF was purchased from Sigma–Aldrich Inc., St. Louis, USA and sulindac (Fig. 1), the internal standard (IS) from Aldrich Chemical Company, Inc., USA. HPLC grade methanol was obtained from J.T. Baker (Phillipsburg, USA), acetonitrile was HPLC grade and was purchased from Merck (Darmstadt, Germany). Ascorbic acid and reagents used for drug extraction were analytical grade and were purchased from Merck (Darmstadt, Germany). The water used was deionised and filtered with a Milli-Q water processing system (Millipore, São Paulo, Brazil). Acetic