Journal of Chromatography B, 975 (2015) 45–51

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Quantitation of the immunological adjuvants, monophosphoryl lipid A and Quil A in poly (lactic-co-glycolic acid) nanoparticles using high performance liquid chromatography with evaporative light scattering detection

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ARTICLE INFO

Article history:
Received 18 July 2014
Accepted 8 November 2014
Available online 18 November 2014

Keywords:
Adjuvants
ELSD
Validation
Nanoparticles
Encapsulation efficiency
In vitro release

ABSTRACT

Monophosphoryl lipid A (MPL) and Quil A are two immunological adjuvants commonly used in vaccines. At present no simple, validated methods for the quantification of Quil A and MPL have been previously reported therefore the aim of the current study was to develop a simple, fast and validated method to quantify MPL and Quil A using high performance liquid chromatography evaporative light scattering detection (HPLC-ELSD). The HPLC-ELSD technique was carried out using a ZORBAX Eclipse XDB-C8 column (2.1 × 50 mm; particle size, 3.5 μm) in an isocratic elution mode at 25 °C. MPL was eluted at a retention time of 1.8 min with methanol-water as the mobile phase and a detector temperature of 75 °C. Quil A was resolved as three peaks with retention times of 4.1, 5.5 and 6.4 min with a detector temperature of 30 °C and with water-acetonitrile and 0.01% formic acid as the mobile phase. The nebulizer pressure and gain were set at 3.5 bar and 10, respectively. Calibration curves plotted for both the adjuvants had an $R^2 > 0.997$. Accuracy, intra- and inter-day precision were within the accepted limits. The limit of detection for MPL and Quil A were calculated as 1.343 and 2.06 μg/mL, respectively. The limit of quantification was 2.445 for MPL and 8.97 μg/mL for Quil A. This analytical method was used to quantify the entrapment and *in vitro* release of MPL and Quil A in a poly lactic-co-glycolic acid (PLGA) nanoparticle vaccine.

1. Introduction

Adjuvants are potent substances that are delivered in combination with antigens in order to modulate and improve immune responses to vaccines and to reduce the amount of antigen or number of booster doses required [1,2]. Monophosphoryl lipid A (MPL) is a potent, non-toxic derivative of lipopolysaccharide from *Salmonella minnesota* (Fig. 1a). It is a Toll-like receptor (TLR)- 4 ligand capable of inducing strong Th1 immune responses [3] and was the first TLR ligand to be approved for commercial use as an adjuvant in the human papilloma virus vaccine (Cervarix™) and the hepatitis B virus vaccine (FENDrix®). MPL has also been evaluated in human clinical trials for malaria, herpes simplex virus, metastatic melanoma, breast cancer and colorectal cancer vaccines [4]. Quil A is a combination of triterpene glycosides (Fig. 1b), extracted from the bark of *Quillaja saponaria molina* [5]. Quil A is a TLR-independent adjuvant and has been shown to induce strong cytotoxic T cell and antibody responses [6,7]. It is widely used in veterinary vaccines and clinical trials are in progress for the development of malaria, cancer (melanoma) and influenza vaccines using QS-21 (a purified component of Quil A). Individually, both MPL and Quil A have shown the ability to improve immune responses, but recent studies suggest a combination of both these adjuvants can stimulate much stronger cellular and humoral responses than each adjuvant separately [8,9].

PLGA nanoparticles have been widely investigated for both drug and vaccine delivery [10,11]. The physicochemical properties of PLGA nanoparticles can be readily modified and the particles can accommodate a wide range of actives [12]. As PLGA nanoparticles have a size similar to pathogens, they are naturally targeted to dendritic cells via phagocytosis and can deliver vaccine components more effectively than a non-particulate vaccine [13].

Chromatographic analysis of MPL and Quil A has been attempted using UV detection, however the lack of chromophores, weak absorbance, low sensitivity and high baseline noise made the analysis difficult [14–17]. Therefore alternatives such as the Limulus Amebocyte Lysate (LAL) endotoxin test to measure MPL have been utilized. While the LAL test is widely used, the assay can be

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http://dx.doi.org/10.1016/j.jchromb.2014.11.006
1570-0232/

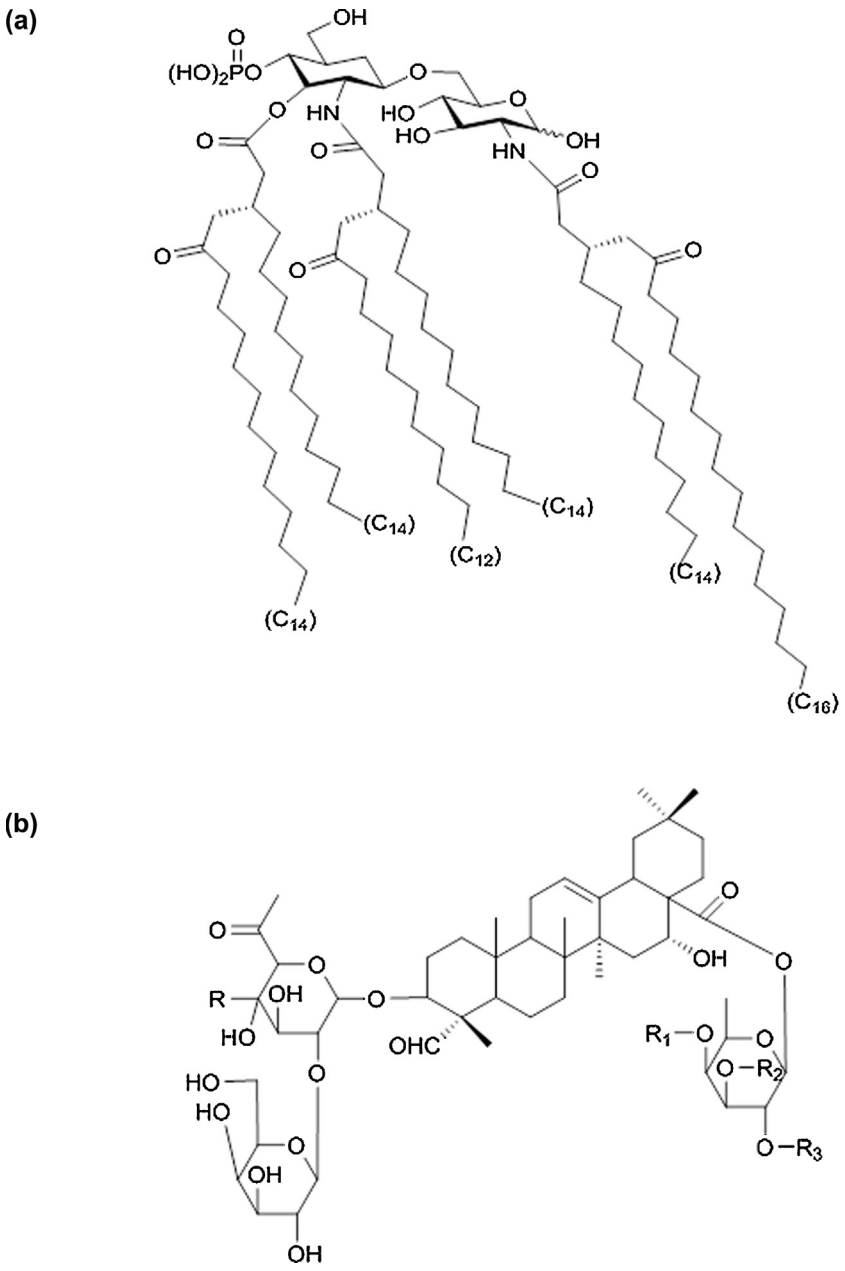

Fig. 1. Chemical structures of (a) MPL [30] and (b) Quil A [20].

technically challenging, is sensitive to laboratory contamination and can produce ambiguous results [18]. Other options involve the derivatization of MPL prior to analysis [16] or the use of mass spectrometry [19,20]. In the present study to overcome these problems we have used high performance liquid chromatography with evaporative light scattering detection (ELSD). ELSD is commonly used as a semi-universal mass detector for HPLC as it can detect analytes by photon scattering without relying on optical properties of the compounds of interest [21,22]. The ELSD technique is sensitive, can detect any analyte less volatile than the mobile phase and is compatible with a wide range of solvents and chromatographic techniques [23].

The aim of this study was to develop a reliable, simple and fast assay to quantify both MPL and Quil A using HPLC-ELSD. The validated assay was then used to investigate the encapsulation efficiency of MPL and Quil A in PLGA nanoparticles and the *in vitro* release of the adjuvants from nanoparticles.

2. Materials and methods

2.1. Materials

Monophosphoryl lipid A from *Salmonella minnesota* RE 595 and formic acid were purchased from Sigma-Aldrich (Missouri, USA). Purified saponin (Quil-A®) was obtained from Brenntag Biosector (Frederikssund, Denmark). PLGA copolymer with a 50:50 monomer ratio and inherent viscosity of 0.55–0.75 dL/g was acquired from Absorbable Polymers International (Pelham, USA). Polyvinyl alcohol (PVA), MW~25,000, was purchased from Polysciences, Inc., (Warrington, USA). HPLC-grade chloroform, methanol and acetonitrile were purchased form Merck KGaA (Darmstadt, Germany). Water was purified using a Millipore® Purification System (USA) and was used during sample preparation and HPLC analysis. Nitrogen gas (oxygen free) was supplied by BOC Ltd., (Auckland, NZ).