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ORIGINAL ARTICLE

Rapid, simple and stability-indicating determination of polyhexamethylene biguanide in liquid and gel-like dosage forms by liquid chromatography with diode-array detection

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Abstract A rapid and simple method for the determination of polyhexamethylene biguanide (polyhexanide, PHMB) in liquid and gel-like pharmaceutical formulations by means of high performance liquid chromatography coupled to diode-array detection (HPLC–DAD) was developed. Best separation was achieved using a cyanopropyl bonded phase (Agilent Zorbax Eclipse XDB-CN column 4.6 mm × 75 mm with particle size of 3.5 μm) as well as gradient elution consisting of acetonitrile/deionized water at a flow rate of 1.0 mL/min. The optimized and applied chromatographic conditions permitted separation of polyhexanide from interacting matrix with subsequent detection at a wavelength of 235 nm with good sensitivity. The method validation was carried out with regard to the guidelines for analytical procedures demanded by the International Conference on Harmonisation (ICH). Mean recoveries of 102% and 101% for gel-like as well as liquid preparations were obtained. Suitable repeatability as well as intermediate precision could be achieved with limits of detection ≤0.004 mg/mL for both formulations, equivalent to ≤0.004% PHMB concerning sample preparation. Determination of PHMB was accomplished without tedious sample preparation. Interacting matrix could be eliminated by the chromatographic

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procedure with excellent performance of system suitability. All analytical requirements were fulfilled permitting a reliable and precise determination of PHMB in pharmaceuticals. Furthermore, the developed method was applied to stability testing of pharmaceutical preparations containing PHMB.

1. Introduction

Polyhexamethylene biguanide hydrochloride (Polyhexanide, PHMB) is a chemical biocide. Therefore, it is used as an active ingredient in a lot of products such as wet wipes, wound irrigation solutions, sterile dressings as well as disinfectants. Due to its excellent properties the usage increased for application to personal care products and pharmaceuticals in wound management, for instance treatment of chronic wounds and burns [1]. This widely used biocide has been reviewed by US Environmental Protection Agency (EPA) and noted, with the exception of occupational users, as having very low aggregate risk of adverse health effects to the public or environment [2].

PHMB binds to the negatively charged phosphate head groups of phospholipids at bacteria cell wall, causing increased rigidity, sinking nonpolar segments into hydrophobic domains, disrupting the membrane with subsequent cytoplasmic shedding culminating in cell death [3]. The antibacterial activity of PHMB depends on the molecular structure. Minimum requirements are met by more than 2 biguanide moieties and 5–7 methylene groups as a spacer. Therefore, PHMB represents an oligomeric substance with number-average degree of polymerization of 2–5 [3]. The chemical structure of PHMB is shown in Fig. 1. It is a cationic biocide marketed worldwide, because of its excellent antimicrobial activity, chemical stability, low toxicity and reasonable cost [4]. PHMB is highly soluble in water (20%, w/v) and aliphatic alcohols but poorly soluble in nonpolar liquids. The biguanide moieties are strong bases and monoprotonated at a pH value of 7 (pKa1 = 2–3; pKa2 = 10.5–11.5) resulting in a polycation with a positive charge at each biguanide moiety [3].

With regard to its application in surgical dressing the need of pharmaceutical preparations containing PHMB on the basis of liquid and gel-like dosage forms increased clearly over the last years in our hospital. Therefore, products from our own manufacture were established for application to our hospital's wards requiring examinations in quality control to ensure the pharmaceutical drug safety. Hence, beside physicochemical specifications, quantification of the PHMB content is essential. For those liquid and gel-like preparations of PHMB a sensitive and specific method of determination should be used. Several methods for analyzing PHMB in chemical disinfectants, eye drops or further personal care products are described in literature [5–12]. Chromatographic applications like liquid chromatography or capillary electrophoresis as well as titrimetric methods were used. Most applications exhibited high limits of detection (LOD) insufficient for the referred applications. In addition, PHMB only shows applicable UV absorption at 235 nm attributed to the π–π^* transition of –C═N– in the biguanide group [4,13] that was used in determination of PHMB in disinfectants by high performance liquid chromatography coupled to diode-array detection (HPLC–DAD) but a high LOD was also observed. Nevertheless, all described methods in literature report on the determination of PHMB in non-pharmaceutical formulations (medical devices as well as personal care products). However, there are no reports of HPLC methods in literature that were developed and validated for the analysis of pharmaceutical formulations as well as stability testing. Moreover, as reported by Al-Rimawi et al. [14], methods of Pharmacopeia for raw material analysis sometimes provide procedures which either do not include pharmaceutical formulations or cannot be applied to them due to missing selectivity concerning degradation products and interfering excipients present in the pharmaceutical dosage forms. In terms of polyhexanide, only one monograph (namely *polihexanide solution* 20% used as a raw material for drug manufacturing as well as extemporaneous products) is listed using a gravimetric analysis which is unsuitable for the specific analysis of polyhexanide in pharmaceutical formulations.

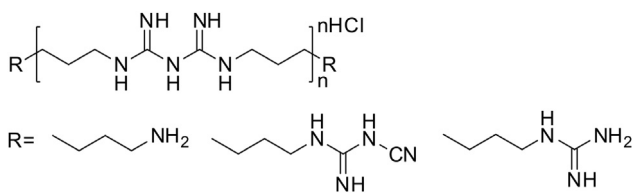

Fig. 1 Chemical structure of PHMB and possible terminal groups.

Thus, the aim of this study was to develop and implement an analytical procedure for PHMB in liquid and gel-like dosage forms with the required sensitivity and specificity for quality control in drug manufacturing. Typical concentrations of PHMB in those formulations are 0.04% (w/w). Liquid chromatography coupled to diode-array detection should be used as a commonly applied system. The guidelines of the International Conference on Harmonisation (ICH) were adopted for method validation [15]. In order to implement an efficient, economic and simple analytical procedure both sample preparation and analysis should be accomplished in one step separating matrix compounds and quantifying the analyte.

This paper presents a rapid, simple and stability-indicating method based on HPLC–DAD that was developed for the separation and quantification of PHMB in liquid and gel-like pharmaceutical dosage forms without tedious sample preparation for the first time.

2. Experimental

2.1. Chemicals and reagents

HPLC-grade water (deionized water) was obtained by purifying demineralized water in a Milli-Q system (Millipore,