Biologicals xxx (2016) 1–8

Contents lists available at ScienceDirect

Biologicals

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

Evaluation of capillary zone electrophoresis for the determination of protein composition in therapeutic immunoglobulins and human albumins

Stefan Christians [†], Nadine Denise van Treel[*], Gabriele Bieniara, Annika Eulig-Wien, Kay-Martin Hanschmann, Siegfried Giess

Paul-Ehrlich-Institut, Federal Institute for Vaccines and Biomedicines, Paul-Ehrlich-Str. 51-59, 63225 Langen, Germany

ARTICLE INFO

Article history:
Received 21 October 2015
Received in revised form
25 February 2016
Accepted 11 April 2016
Available online xxx

Keywords:
Capillary zone electrophoresis
Protein composition
Immunoglobulins
Albumin
Human serum
European pharmacopoeia

ABSTRACT

Capillary zone electrophoresis (CZE) provides an alternative means of separating native proteins on the basis of their inherent electrophoretic mobilities. The major advantage of CZE is the quantification by UV detection, circumventing the drawbacks of staining and densitometry in the case of gel electrophoresis methods. The data of this validation study showed that CZE is a reliable assay for the determination of protein composition in therapeutic preparations of human albumin and human polyclonal immunoglobulins. Data obtained by CZE are in line with "historical" data obtained by the compendial method, provided that peak integration is performed without time correction. The focus here was to establish a rapid and reliable test to substitute the current gel based zone electrophoresis techniques for the control of protein composition of human immunoglobulins or albumins in the European Pharmacopoeia. We believe that the more advanced and modern CZE method described here is a very good alternative to the procedures currently described in the relevant monographs.

1. Introduction

Human immunoglobulins as defined by the European Pharmacopoeia (Ph. Eur.) are purified polyclonal antibodies derived from human blood and are described according to their mode of administration in the monographs 0338 [1], 0918 [2], and 2788 [3]. Therapeutic albumins, too, are derived from human blood and are described as human albumin solution in monograph 0255 [4].

One of the major quality parameters in albumins and immunoglobulins is the purity with respect to the protein composition. The determination of the protein composition is the major purity test for therapeutic immunoglobulins as well as for therapeutic albumins. Thus the relevant monographs require an examination by zone electrophoresis (ZE) for all polyclonal immunoglobulins and albumins [1–4]. Currently, electrophoresis is to be performed as a classical ZE using either cellulose acetate or agarose gels as supporting material. After electrophoresis the proteins are stained with amido black 10B and quantified by densitometry [1–4]. Although this technique has been established successfully by many manufacturers and regulatory control laboratories, it has some major drawbacks. It is relatively labour intensive with a low potential for automation (Electrophoresis time: 25–35 min; Staining time: app. 2 h; Quantification by densitometry: 30 min). Therefore conventional gel electrophoresis is not suitable as a high throughput method. From an analytical point of view a more serious disadvantage is presented by the fact that integration limits

Abbreviations: Ac-Trp, N-acetyl tryptophan; Albumin-BRP2, Human albumin for electrophoresis BRP Batch 02; Albumin-BRP3, Human albumin for electrophoresis BRP Batch 03; BRP, Biological reference preparation; CA, Cellulose acetate; CE, Capillary electrophoresis; CI, Confidence interval; CV, Coefficient of variation; CZE, Capillary zone electrophoresis; DOE, Design of Experiments; EDQM, European Directorate for the Quality of Medicines; ICH, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; i.d., inner diameter; IG-BRP2, Human immunoglobulin for electrophoresis BRP Batch 02; IG-BRP3, Human immunoglobulin for electrophoresis BRP Batch 03; IGIV, Human immunoglobulin for intravenous administration; LOQ, Limit of quantification; Ph. Eur., European Pharmacopoeia; RSD, Relative standard deviation; SD, Standard deviation; TCA, Time corrected area; ZE, Zone electrophoresis.

* Corresponding author.
E-mail addresses: nadine.vantreel@pei.de (N.D. van Treel), gabriele.bieniara@pei.de (G. Bieniara), annika.eulig-wien@pei.de (A. Eulig-Wien), kay-martin.hanschmann@pei.de (K.-M. Hanschmann), siegfried.giess@pei.de (S. Giess).
[†] Deceased.

http://dx.doi.org/10.1016/j.biologicals.2016.04.001
1045-1056/

as well as baseline corrections have to be set manually. This makes the test prone to a significant personal bias. Capillary zone electrophoresis (CZE), however, combines the resolution power of electrophoresis with the reliability of quantification typical for HPLC-techniques. CZE performed under appropriate conditions in a suitable fused silica capillary leads to a separation similar to the gel zone electrophoresis. UV-detection allows the direct quantification of proteins without prior staining.

Although capillary zone electrophoresis is already in use in the field of clinical chemistry, the aim of this study was to provide a simple, reliable CZE method appropriate as a test in Ph. Eur. monographs. To this purpose it is desirable to forego any proprietary components, such as capillary coatings or buffer additives. Furthermore, results should be directly comparable to results obtained by the current Ph. Eur. methods, whereas achievements like maximum resolution or minimum limits of detection are only minor goals here.

2. Material and methods

2.1. Compendial method

Conventional ZE using either cellulose acetate or agarose as supporting gel were used as reference methods. These methods were performed by exactly following the instructions of the Ph. Eur. monograph for human immunoglobulins for intravenous administration [2].

2.2. Capillary zone electrophoresis (CZE)

2.2.1. Equipment and software

The study was performed using different CE systems from Beckman Coulter GmbH (Now: SCIEX), Germany: P/ACE MDQ, PA-800enhanced and PA-800plus were all equipped with a UV-Detector. The systems were controlled by Beckman–Coulter 32Karat™-Software Version 6.8 (P/ACE MDQ) or 7.0 (for PA800 enhanced and PA-800plus Systems). The UV-signal was recorded using an A/D-converter (e-Sat/in™, Waters GmbH, Germany) and evaluated with Waters Empower™ Software Version 2.0.

2.3. Samples and reference material

Various therapeutic human immunoglobulins for intravenous administration (IGIV) and therapeutic human albumins and mixtures thereof were used as samples. All of these therapeutics are currently licensed in the EU.

Human Immunoglobulin for Electrophoresis BRP Batch 02 was used as a reference preparation. This reference preparation was supplied by the European Directorate for the Quality of Medicines (EDQM) and consisted of a lyophilized human immunoglobulin preparation with a purity of 75.7%–80.9% γ-globulin [5]. The abbreviation “IG-BRP2” will be used to refer to this reference preparation.

Human Immunoglobulin for Electrophoresis BRP Batch 03 was also used as a reference preparation. It is just another batch of the former IG-BRP02. The declared purity of this batch is 79.8%–86.4% γ-globulin [5]. The abbreviation “IG-BRP3” will be used to refer to this reference preparation.

Human Albumin for Electrophoresis BRP Batch 02 was used as a reference preparation for albumins. This reference preparation is also supplied by the European Directorate for the Quality of Medicines (EDQM) and consists of a lyophilized human albumin preparation with a purity of 96.7%–99.2% albumin [6]. The abbreviation “Albumin-BRP2” will be used to refer to this reference preparation.

Human Albumin for Electrophoresis BRP Batch 03 was also used as a reference preparation for albumins and is the replacement batch of the former Albumin-BRP2. The declared purity of this batch is 93.8%–98.3% albumin [6]. The abbreviation “Albumin-BRP3” will be used to refer to this reference preparation.

Human normal sera were obtained as samples provided for interlaboratory trials organized by Instand e.V., Germany. Instand e.V. is a professional provider of collaborative studies for the assessment of quality and performance of medical laboratories.

SPE-01 and *SPE-02* are in-house quality control standards which were derived from licensed human immunoglobulins spiked with a significant amount of human albumin as artificial impurity. The preparations are lyophilized and reconstituted daily before use.

2.4. Sample preparation

If not stated otherwise sample preparation simply consisted of dilution with running buffer to a final protein concentration of 10 mg/mL. When UV absorbing excipients were expected to interfere with the electropherograms, samples were ultrafiltrated using Amicon Ultra Filters 0.5 mL with a molecular weight cut-off of 10 kDa (Millipore, Germany) according to manufacturer's instructions prior to dilution with 300 mM borate buffer.

2.5. Electrophoretic conditions

A 300 mM borate running buffer was prepared by dissolving 18.6 g boric acid into HPLC grade water, adjusting the pH to 9.0 with NaOH and filling to 1000.0 mL with water. The buffer was filtered through 0.22 μm filters and degassed by sonication for 10 min. The buffer is stored in polypropylene bottles at room temperature for up to 1 year.

Electrophoresis was performed in fused silica capillaries (i.d.: 50 μm; total length: 40 cm; length to window: 30 cm). The detector was set to 214 nm and 4 Hz, the sample storage and cartridge were temperated at 20 °C. Prior to each injection the capillary was rinsed at 50 psi with 1 N NaOH for 1 min, with water for 1 min and with running buffer for 2 min. Immediately before and after each injection a short dip of the capillary ends in water was included to prevent sample or buffer carry over.

The diluted samples were injected hydrodynamically for 5 s with 0.5 psi. Electrophoresis was performed with 12.0 kV (300 V/cm) at normal polarity for 15 min. Capillary cleaning after each run consisted of rinsing at 50 psi with 0.1 N HCl for 1 min and water for 2 min.

During method development it was found that the evaluation of peak area percent was more appropriate than the evaluation of time corrected peak areas. This will be discussed below.

2.6. Test conception, statistical analyses, and acceptance criteria

2.6.1. Validation strategy

According to the ICH guideline on validation of analytical procedures [7] the determination of protein composition of immunoglobulins is considered to be a quantitative test for impurities, requiring the validation of accuracy, specificity, repeatability, intermediate precision, limit of quantitation, linearity, and range.

2.6.2. Accuracy

2.6.2.1. Accuracy using Ph. Eur. biological reference preparations. Immunoglobulins: The accuracy of immunoglobulins was determined by analysing the biological reference preparation IG-BRP2 and IG-BRP3 provided by EDQM. The reference preparations were analysed 3 times and the resulting data were compared to the acceptable ranges, which were obtained by EDQM in

Please cite this article in press as: Christians S, et al., Evaluation of capillary zone electrophoresis for the determination of protein composition in therapeutic immunoglobulins and human albumins, Biologicals (2016), http://dx.doi.org/10.1016/j.biologicals.2016.04.001