Continued improvements in the quality and consistency of follitropin alfa, recombinant human FSH

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Abstract

The use of gonadotrophins for the treatment of infertility began in the 1930s following early work on the pituitary–ovarian axis and the discovery of FSH and LH. The technological development of pharmaceutical gonadotrophins over the last 40 years has shown improvements in specific activity, purity, degradation and impurities. Throughout these pharmaceutical developments the gonadotrophin content of both urinary and recombinant preparations has been assessed using an animal in-vivo bioassay. This paper reflects upon the manufacturing history of recombinant human FSH (r-hFSH) and follitropin alfa filled by mass (FbM), and evaluates the impact of introducing a pharmaceutical product that is formulated and assayed by a physicochemical method for r-hFSH protein content. It also compares the analytical consistency of follitropin alfa FbM with another commercially available r-hFSH, follitropin beta.

Keywords: consistency, filled-by-mass, follitropin alfa, isoforms, purity, recombinant human FSH

Introduction

The use of gonadotrophins for the treatment of infertility began in the 1930s following early work on the pituitary–ovarian axis and the discovery of FSH and LH (Ludwig et al., 2002; Lunenfeld, 2002). Early preparations were derived from animal sources, pituitaries and pregnant mare serum, or from the pituitary glands of human post-mortem origin. These presentations were abandoned due to safety concerns. However, gonadotrophins extracted from urine proved to be effective for ovarian stimulation and generally well tolerated. The urinary gonadotrophins were used universally until the introduction of recombinant gonadotrophins in the 1990s. Today, recombinant gonadotrophins are widely recognized as the gold standard.

The technological development of pharmaceutical gonadotrophins over the last 40 years has shown improvements in specific activity, purity, degradation and impurities. This development has been largely due to improved manufacturing and purification processes, and the introduction of recombinant technology. Improvements in technology have been associated with an increased degree of control over variables affecting the likelihood of a successful outcome. The first urinary gonadotrophin, human menopausal gonadotrophin (HMG), contained a mixture of FSH and LH activity, together with miscellaneous urinary proteins. As discussed below, pharmaceutical development resulted in the production of highly purified urinary FSH (u-FSH-HP), with residual levels of LH and urinary derived proteins. Recombinant human FSH (r-hFSH) has still higher purity and specific activity compared with u-FSH. Meta-analysis of clinical trials has shown a superior efficacy in terms of higher pregnancy rate in in-vitro fertilization cycles (overall 3.7%) using r-hFSH compared with those in which u-FSH was used (Daya, 2002).

The recent availability of recombinant human LH (r-hLH) as a separate preparation has further increased control over the treatment cycle by allowing clinicians to ensure that
exogenous LH is administered only in the treatment of those patients who require it; for example, in patients with anovulatory infertility due to hypogonadotrophic hypogonadism (World Health Organization type I) (European Recombinant Human LH Study Group, 1998), or in patients of older reproductive age (Humaidan et al., 2004; Marrs et al., 2004). Control over LH levels during assisted reproduction and ovulation induction is important because of evidence for an ‘LH window’ (Hillier, 1994). In the absence of a threshold concentration of serum LH, oestradiol production will be insufficient for follicular development and endometrial proliferation. However, exposure of the developing follicle to excessive LH may result in atresia and cessation of normal development (Balasch and Fabregues, 2002; Shoham, 2002; Loumaye et al., 2003).

These examples illustrate how developments in manufacturing of gonadotrophins have improved control over the treatment cycle and the outcome of treatment in clinical practice. Throughout these pharmaceutical developments, the gonadotrophin content of both urinary and recombinant preparations has been assessed using an animal in-vivo bioassay. More recent analytical improvements have allowed recombinant gonadotrophins to be assessed by precise physicochemical methods.

This paper reflects upon the manufacturing history of r-hFSH and follitropin alfa filled by mass (FbM), and evaluates the impact of introducing a pharmaceutical product that is formulated and assessed on r-hFSH protein content. It also compares the analytical consistency of follitropin alfa FbM with another commercially available r-hFSH, follitropin beta.

**Historical perspective**

Over the last 40 years, there have been many developments in the pharmaceutical manufacture of gonadotrophins (Table 1).

The developments started with the demonstration that gonadotrophins could be extracted from the urine of menopausal women (Katzman et al., 1943). This work led to the commercialization of the first pharmaceutical, urine-derived gonadotrophin, HMG, in 1962 (Donini and Montezemolo, 1949). HMG was classified as a menotrophin and contained a fixed 1:1 ratio of FSH and LH. However, the drug preparation was relatively impure, with a total gonadotrophin content of approximately 2–5% (Giudice et al., 1994). The manufacturing process of HMG required a substantial urine collection network, as 75 IU of the HMG was extracted from approximately 2 litres of urine (Lunenfeld, 2002).

The development of gonadotrophins continued, with the objective of improving their purity and providing a product free of LH and other urine-derived proteins. Two further urinary derived products became available, u-FSH in 1983 (urofollitropin), which had a FSH purity of approximately 5%, and u-FSH-HP in 1993 (highly purified urofollitropin), with an FSH purity of approximately 95% (Giudice et al., 1994). In more recent years, other urine-derived gonadotrophin pharmaceutical preparations, highly purified HMG (HMG-HP), have been developed with a total gonadotrophin content of approximately 70% (Giudice et al., 1994; van der Weijer et al., 2003).

All of the urine-derived gonadotrophin products are subject to drug product description and requirements, as published in international pharmacopoeias (British [BP], European [EP], Unites States Pharmacopoeias [USP]). In all cases, the assessment of the gonadotrophin content is by the ‘in-vivo’ bioassay (Steelman and Pohley, 1953). The in-vivo rat bioassay is an imprecise analytical tool, as it shows a variability of 10–20% (Driebergen and Baer, 2003), and is highly dependent upon the quality and number of the animals used, and upon the skill of the technician in removing the

Table 1. Development of gonadotrophins over the last 40 years.

<table>
<thead>
<tr>
<th>Product</th>
<th>Trade name</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMG</td>
<td>Pergonal</td>
<td>Serono&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Humegon</td>
<td>Organon</td>
</tr>
<tr>
<td></td>
<td>Menogon; Repronex</td>
<td>Ferring&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>HMG-HP</td>
<td>Menopur</td>
<td>Ferring</td>
</tr>
<tr>
<td>u-FSH</td>
<td>Metrodin</td>
<td>Serono</td>
</tr>
<tr>
<td></td>
<td>Follegon</td>
<td>Organon&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Bravelle</td>
<td>Ferring</td>
</tr>
<tr>
<td>u-FSH-HP</td>
<td>Metrodin HP; Fertinex</td>
<td>Serono</td>
</tr>
<tr>
<td>r-hFSH</td>
<td>Follitropin alfa</td>
<td>GONAL-f Serono</td>
</tr>
<tr>
<td></td>
<td>Puregon; Follistim</td>
<td>Serono</td>
</tr>
<tr>
<td>r-hLH</td>
<td>Luveris</td>
<td>Serono</td>
</tr>
<tr>
<td>r-HCG</td>
<td>Ovidrel; Ovitelle</td>
<td>Serono</td>
</tr>
</tbody>
</table>

<sup>a</sup>All trade names listed are registered trade marks.<br>
<sup>b</sup>Serono International SA, 15 bis, chemin des Mines, Geneva, Switzerland.<br>
<sup>c</sup>Ferring Pharmaceuticals, Copenhagen, Denmark.<br>
<sup>d</sup>Organon International Inc., Roseland, NJ, USA.

HCG = human chorionic gonadotrophin; HMG = human menopausal gonadotrophin; HP = highly purified; r-hFSH = recombinant human follicle-stimulating hormone; r-hLH = recombinant human luteinizing hormone; u-FSH = urine-derived FSH.
ovaries from a large number of animals. Furthermore, control of the menopausal urine and the drug substance is limited. Analytical assessment of many of these urine-derived gonadotrophins has demonstrated that they are contaminated by many other naturally occurring proteins present in urine (Giudice et al., 1994, 2001; van der Weijer et al., 2003; Yarram et al., 2004).

The pharmaceutical manufacturing approach of extracting a naturally occurring, clinically useful drug is not limited to gonadotrophins. Insulin (FDA Approval, 1939) (Galloway and Chance, 1994), growth hormone (Raben, 1957), and factor VIII have also been developed as pharmaceutical products and have been extracted from the pig pancreas, human pituitary and human blood respectively. However, all of these pharmaceutical products relied upon the collection of animal- or human-derived tissue, organs or fluids. The control and safety of the source material was less than ideal, as it was eventually demonstrated that certain infectious agents could contaminate the source materials (HIV transmission from blood, CJD from pituitaries; Committee on Growth Hormone Use of the Lawson Wilkins Pediatric Endocrine Society, 1985; Lee, 1998). Furthermore, the increasing demand for urine-derived gonadotrophins had a concomitant effect on the demand for the source material (Gordon, 2002; Lunenfeld, 2002).

The discovery of recombinant DNA technology (Cohen et al., 1973) provided another approach to the pharmaceutical manufacture of complex proteins. In this case, the DNA that coded for the naturally occurring protein was introduced into the genome of a cell. The genetically constructed cell line became the immortal source of the coded protein. The DNA technology led to the development of many highly pure therapeutic proteins through cell fermentation and sophisticated purification procedures. Pharmaceutical companies then rapidly employed DNA technology to develop and manufacture recombinant DNA-derived proteins (Recombinant Insulin FDA Approval, 1982). For the first time the pure form of r-hFSH could be fully characterized (Loumaye et al., 1995). Additionally, developments in analytical methodology also allowed a more extensive assessment of the product throughout the production process. In 1995, the first r-hFSH was made available, and was classified as follitropin alfa (r-hFSH, Howles, 1996), and was followed by the second r-hFSH (follitropin beta; Olijve et al., 1996). Analytical tests could then be used to demonstrate purity, to quantify aggregation, dissociated subunits, and the oxidized alfa subunit, and to demonstrate the glycosylation and isoform pattern of all manufactured batches (Bagatti et al., 2001; Gervais et al., 2003).

Interestingly though, the FSH content of these recombinant proteins was still assessed with an ‘in-vivo’ rat bioassay. As described above, the bioassay analytical method has been recognized as imprecise, costly, and remains under political and ethical pressures due to the use of animals (Mulders et al., 1997). Furthermore, animal testing is widely discouraged when alternative solutions exist (European directive 86/609, art. 7.2; Castle, 1998; Artiges, 1999). The European Pharmacopoeia has continued to review the use of animals in drug product testing and has adopted the scientific principles described in the Technical Guide for the elaboration of the Monograph for Biologicals (4th edition, 2003): ‘...where the battery of physico-chemical tests has been shown to adequately characterize the molecule, a physico-chemical assay alone may be employed’. The International Conference of Harmonization (ICH, guideline Q6B) also confirmed the need for a physico-chemical assay: ‘...quantity, usually measured as protein content, is critical for a biotech product and should be determined by an appropriate assay, usually physico-chemical in nature’.

In fact, a review of the European Pharmacopoeia (4th edition, 2002) documents the use of in-vivo bioassays for only four protein hormones, erythropoietin (EPO), human chorionic gonadotrophin (HCG), human LH and human FSH. It is interesting to note that some of these protein hormones have been assessed using physicochemical analytical methods, with high-performance liquid chromatography (HPLC) protein assays for r-HCG, r-LH, and N-glycan charge assay for EPO (Hermentin et al., 2002).

In contrast, manufacturers of other recombinant proteins had developed physicochemical analytical methods for quantifying the active component or protein content that met the requirements of regulatory authorities (Seamon, 1998). A physicochemical quantification approach is not immediately apparent for r-hFSH and other glycosylated proteins (Bristow and Charton, 2002). The protein is glycosylated, and the degree of sialylation is affected by the cell culture conditions. The sialylation imparts a negative charge on the r-hFSH molecule, which gives rise to a heterogeneous molecule consisting of many different isoforms (Olijve et al., 1996; Gervais et al., 2003). All of the isoforms are recognized by the ‘in-vivo’ rat bioassay, but have differing pharmacokinetic half-lives in humans (Ulloa-Aguirre et al., 1988). Therefore, the heterogeneous glycoprotein FSH did not immediately appear to be a good candidate for introducing alternative physicochemical analytical methods to assess the FSH content.

However, assessment of more than 100 batches of the r-hFSH drug substance (follitropin alfa) did indeed demonstrate that the specific activity, isoform pattern by isoelectric focusing (IEF) and sialylation pattern by glycan mapping were consistent (Driebergen and Baer, 2003). This observation allowed the development of a physicochemical analytical method for measuring the FSH content (by mass of protein) for the product, which was traditionally assessed in bioactivity content. The physicochemical method for assessing the FSH content can be successfully used to assess the r-hFSH content in the active drug substance and the drug product (follitropin alfa FbM). The final drug product is formulated based on the r-hFSH protein content, thus ensuring a more precise r-hFSH content per vial.

Another physicochemical approach to assess the r-hFSH content (follitropin beta) is based upon the isoform pattern of the drug (Mulders et al., 1997; Storring et al., 2002). The analytical approach from Mulders et al. (1997) has demonstrated that the isoform pattern of the drug substance can be directly linked to the level of bioactivity, and thus be used to formulate the drug product. Unfortunately, it appears that the IEF physiochemical method has not been applied to the drug product, and the FSH content in the final product is verified with the traditional bioassay (Follitropin Beta European Product Monograph, 2003).
This paper will present all of the manufacturing data on batches of r-hFSH produced during 1997–2003 and batches of follitropin alfa FbM produced in 2003. The batch-to-batch consistency of the follitropin alfa FbM process is also compared with another commercially available r-hFSH product, follitropin beta.

Materials and methods

Batches of r-hFSH, follitropin alfa, drug substance manufactured between 1997 and 2004 and follitropin alfa (FbM), 75 IU, manufactured between 2003 and 2004 were assessed analytically using the methods described below. Follitropin alfa (FbM), 75 IU, is presented as a lyophilized powder in a 3 ml glass vial. The contents of the vial must be reconstituted with 1 ml of water for injection prior to administration.

For comparison, 10 batches of another commercially available r-hFSH preparation (follitropin beta, solution for injection) were also assessed. Follitropin beta is presented as a solution of r-hFSH in a cartridge. The cartridge must be installed in a pen device prior to administration. Specific activity, isoform pattern, level of sialylation, dissociated subunits, oxidized alpha subunits and protein content of the formulated drug product were determined.

Analytical methods

Size-exclusion high-performance liquid chromatography (SE-HPLC) for r-hFSH protein content

Chromatography was performed on an SE-HPLC column (Biosep S2000 column; Tosoh Bioscience GmbH, Stuttgart, Germany) at room temperature, using a buffer of sodium phosphate and sodium sulphate, pH 6.7. Protein content was determined by comparison against a standard reference calibrated by amino acid analysis.

In-vivo bioasssay for r-hFSH biological activity

Female rats, aged 21 days, were injected with r-hFSH and HCG over 3 days. On day 4, the rats were killed and their ovaries removed and weighed. The in-vivo bioactivity was then evaluated according to the USP and EP.

IEF for r-hFSH isoform pattern distribution

A commercial polyacrylamide gel (ExcelGel 15% polyacrylamide gel; Amersham Biosciences UK Limited, Little Chalfont, Buckinghamshire, UK) was prepared with a solution containing appropriate carrier ampholytes to create a pH gradient of 2.5–6.5. The separated proteins were transferred onto a nitrocellulose membrane, fixed using primary and secondary antibodies, and visualized with an enhanced chemiluminescence method.

Glycan mapping for r-hFSH levels of sialylation

The intact glycan species were released from the core peptides of the r-hFSH, then labelled with a fluorescent marker and separated by anion exchange chromatography according to their charge. This allows the quantification of the glycan species based on the level of sialylation.

Formulated drug products were initially purified by size-exclusion chromatography to remove excipients prior to glycan mapping.

Reverse phase HPLC (RP-HPLC) for the level of oxidized forms of r-hFSH alfa

Chromatography was performed on a C4 column, at 50°C, using a linear gradient of acetonitrile in triethylammonium phosphate, pH 6.0. The two subunits of r-hFSH were separated, and the peaks relative to oxidized alpha subunit were quantified as a proportion of the intact subunit.

SDS-polyacrylamide gel electrophoresis for r-hFSH dissociated subunit content

An 8–18% gradient acrylamide gel electrophoresis was used to assess subunits of r-hFSH. Free subunits were visually detected using a silver stain and quantified by scanning densitometry.

SDS-polyacrylamide gel electrophoresis for r-hFSH aggregate content

An 8–18% gradient acrylamide gel electrophoresis was used to assess aggregates of r-hFSH. Aggregates were visually detected using a silver stain and quantified by scanning densitometry.

Results

r-hFSH, follitropin alfa, drug substance

Specific activity

The specific activity is the ratio of the biological activity (IU, bioassay) and the protein content (mg, protein by SE-HPLC). Figure 1 shows the mean specific activity for 309 batches manufactured between 1997 and 2003. The mean specific activity over this period was 13,645 IU/mg.

The data demonstrate that the specific activity of follitropin alfa is normally distributed, unaffected by bioreactor run, and stable over many years of manufacturing. The between-batch variability is attributed to the imprecise assessment of the bioactivity (Driebergen and Baer, 2003).

The data clearly demonstrate a consistency in the specific activity over 7 years of r-hFSH follitropin alfa manufacturing history. Similar data have been previously presented (Driebergen and Baer, 2003) for r-hFSH batches manufactured from 1997 to 2000. The mean specific activity over the 7-year period is 13,645 IU/mg, with a variability of 8.5% and a mean mass of 5.50 µg protein, equivalent to 75 IU, with a variation between years of 2.7%.

The consistency of specific activity demonstrates the consistent relationship between r-hFSH protein content and bioactivity, and so allows the r-hFSH content of follitropin alfa to be assessed in mass (protein content).
**Isoform pattern**

The isoform pattern and relative quantification of r-hFSH, follitropin alfa, was assessed in 173 batches manufactured from 2000 to 2003. The IEF of r-hFSH produced seven major bands at pI values between 4.20 and 5.05. Five minor bands were also seen between pI values of 5.25 and 6.30, and one minor band at pI 4.1. The relative proportions of each isoform are shown in Figure 2.

The data demonstrate a consistency in the r-hFSH follitropin alfa isoform distribution over the last 4 years of manufacturing history. Similar data have been previously presented (Driebergen and Baer, 2003) for 63 r-hFSH batches manufactured from 1999 to 2000.

**Glycan mapping**

The glycan mapping pattern and relative quantification of the glycan species based on the amount of sialylation in r-hFSH follitropin alfa was assessed in 163 batches manufactured from 2000 to 2003 (Figure 3).

The di-sialylated glycans were the most common (mean 49.5 ± 1.3), followed by the mono- (mean 24.1 ± 2.1), tri- (mean 16.0 ± 1.8), and tetra-sialylated (mean 4.7 ± 1.3) forms. The neutral glycans accounted for 5.7 ± 1.4 of the total.

The data demonstrate a consistency in the amount of sialylation of the glycan species in r-hFSH follitropin alfa over 4 years of manufacturing history. Similar data was previously presented (Driebergen and Baer, 2003) for 121 r-hFSH batches manufactured from 1998 to 2000.

**Degradation pattern: oxidation of the alpha subunit, dissociated subunits and aggregates**

The degradation of the r-hFSH follitropin alfa molecule was assessed by measuring the relative amounts of the oxidized alpha subunit, free dissociated subunits and aggregates in 173 batches manufactured between 2000 and 2003. The batches contained a mean of 1.6% oxidized alpha subunit (range <1–2.4%), <3% dissociated subunits and <1% aggregates.

The results indicate that the r-hFSH molecule has a consistent high level of purity, and very low level of degraded r-hFSH in the drug substance.

**Follitropin alfa FbM, 75 IU**

**Protein content**

The protein content of follitropin alfa FbM was assessed by SE-HPLC in 30 batches. This analytical method has a very high level of inter-laboratory precision, 1–2% (Driebergen and Baer, 2003). The mean protein content measured was 6.1 µg per vial compared with the nominal target of 6.0 µg per vial (Figure 4). The

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**Figure 1.** Variation in specific activity of recombinant human follicle-stimulating hormone (r-hFSH) follitropin alfa manufactured between 1997 and 2003.

**Figure 2.** Variability in isoelectric focusing (IEF) pattern of recombinant human FSH (r-hFSH) follitropin alfa.

**Figure 3.** Variation of the glycan map of recombinant human FSH (r-hFSH) follitropin alfa (163 batches).

**Figure 4.** The protein content (µg/75 IU vial) of 30 batches of recombinant human FSH (r-hFSH) follitropin alfa filled by mass.
variability of the protein content over the 30 batches was ± 1.6%.

**Degradation levels in follitropin alfa FbM**

The relative degradation of follitropin alfa FbM was assessed by measuring the relative amounts of oxidized alpha subunit, free dissociated subunits and aggregates.

The degradation level was assessed in 30 consecutive batches of follitropin alfa FbM manufactured in 2003–2004. The mean percentage of oxidized subunits was 1.7% (range 1.0–2.5%), while dissociated subunits made up <5% and aggregates <2%, indicating that the r-hFSH has a consistent high level of purity and a low level of r-hFSH degradants in the drug product.

**Isoform pattern in follitropin alfa FbM**

The isoform pattern and relative quantification of follitropin alfa FbM was assessed in nine batches manufactured in 2003 from different batches of r-hFSH drug substance. The results demonstrate a highly consistent isoform pattern (Figure 5).

**Follitropin beta, solution for injection**

Ten batches of another commercially available r-hFSH preparation (follitropin beta, solution for injection) were analysed to assess the relative quality and manufacturing consistency.

**Degradant level and specific activity**

Each batch of commercial product was assessed for protein content, specific activity, percentage of oxidized alpha FSH, percentage of dissociated subunits (Table 2), glycan map and isoform distribution.

The level of degraded r-hFSH in follitropin beta solution for injection, assessed as oxidized alpha subunit, was between 3.5 and 5.3%, which is slightly higher than routinely seen with follitropin alfa FbM (maximum 2.5%). The level of dissociated subunits was always less than 3%.

The specific activity of follitropin beta solution for injection varied from 7230 to 10,392 IU/mg, with a mean specific activity of 9396 IU/mg, comparable with earlier published data (de Leeuw et al., 1996; Mulders et al., 1997; Horsman et al., 2000). The data show that the specific activity of follitropin beta is more variable and has a lower bioactivity to protein ratio than that of follitropin alfa FbM. The difference in specific activity may be related to the distribution of the isoforms in follitropin beta.

The more variable specific activity seen in batches of follitropin beta has also been calculated as protein content per 75 IU. The mean protein content per 75 IU was 8.06 µg and the between-batch variability (% CV) in protein content for follitropin beta was 12%. In contrast, the between-batch variability in protein content for follitropin alfa FbM was 1.6% (see above).

**Glycan mapping**

The levels of sialylation of the glycan species in follitropin beta solution for injection are similar to those found in r-hFSH, follitropin alfa and as previously published (Olijve et al., 1996). Di-sialylated glycans were most common (mean 45.1 ± 0.8%), followed by mono- (23.3 ± 2.5%), tri- (19.8 ± 1.5%) and tetra-sialylated (7.2 ± 1.2%) glycans and neutral glycans (4.6 ± 0.8%).

**Isoform pattern**

For comparative purposes the isoform pattern, as analysed by IEF, of follitropin beta solution for injection and follitropin alfa FbM is shown in Figure 6. The consistency of the isoform pattern for follitropin alfa FbM has already been discussed. The IEF gels of the follitropin beta batches tended to show more individual variability in isoform pattern, with less consistent profile in the basic isoforms (Figure 6a, lanes 3, 4 and 5) and were less consistent than the isoform profile of follitropin alfa FbM.

![Figure 5. Isoform pattern of nine batches of follitropin alfa filled by mass (FbM). Lane 1, pI markers; lanes 2–10, batches of follitropin alfa (FbM).](image_url)
### Table 2. Analytical data for follitropin beta.

<table>
<thead>
<tr>
<th>Batch</th>
<th>Dissociated subunits (%)</th>
<th>Oxidized product (%)</th>
<th>Protein content (µg/ml)</th>
<th>Specific activity (IU/mg)</th>
<th>Protein content (µg) in 75 IU</th>
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<tr>
<td>600 IU, 310870</td>
<td>&lt;3</td>
<td>3.5</td>
<td>67.5</td>
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<td>% CV</td>
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**Figure 6.** Isoform patterns of (a) follitropin beta: lane 1, pI markers; lanes 2–11, follitropin beta solution for injection batches [1 µg recombinant human FSH (r-hFSH (added)] and (b) follitropin alfa filled by mass (FbM): lane 1, pI markers; lanes 2–10, follitropin alfa FbM batches (20 IU r-hFSH loaded).
Discussion

As discussed previously by Driebergen and Baer (2003), the physicochemical consistency of the r-hFSH manufacturing process (follitropin alfa) has now been demonstrated over 7 years of routine manufacture. The physicochemical consistency has resulted in a consistent specific activity (IU/mg protein) that has allowed the assessment of r-hFSH content (follitropin alfa) by a highly precise and accurate SE-HPLC method rather than the highly imprecise in-vivo rat bioassay. The assessment of the r-hFSH content using the SE-HPLC assay has now been translated into the final pharmaceutical form, follitropin alfa FbM. The r-hFSH content of follitropin alfa FbM can now be assessed by the same highly precise and accurate SE-HPLC assay, and has demonstrated a significantly improved between-batch consistency. The consistency in r-hFSH content of follitropin alfa FbM was also compared with another commercially available r-hFSH product (follitropin beta, solution for injection), and the relative variation between batches was 1.6% for follitropin alfa FbM (n = 30) and 12% for follitropin beta solution for injection (n = 10).

Alternative physicochemical techniques have been described to assess the r-hFSH content (follitropin beta) in the active drug substance (Mulders et al., 1997; Storring et al., 2002) by measuring the isoform distribution of r-hFSH by IEF. However, the analytical approach is not used to assess the r-hFSH content of the final pharmaceutical form (follitropin beta, solution for injection). The FSH content of the final pharmaceutical form continues to be assessed using the less precise in-vivo rat bioassay. The analytical assessment of r-hFSH content (follitropin beta) by IEF has a variation of about 4% (Storring et al., 2002) and follitropin beta solution for injection, by rat bioassay, has a variation of 10% (Mulders et al., 1997; Driebergen and Baer, 2003). The combined analytical variation shown for follitropin beta can be directly compared with the low analytical variation for follitropin alfa FbM (1.2%).

The additional analytical assessment of r-hFSH has demonstrated the high purity of the pharmaceutical presentation and the low levels of degradation. The superior purity of r-hFSH remains in contrast to the FSH purity and presence of contaminating urinary proteins still seen in commercially available, urine-derived gonadotrophins.

Several clinical studies have been undertaken to assess the clinical implications of the physicochemical improvements of follitropin alfa FbM. These studies have demonstrated that the use of follitropin alfa FbM provided an improvement in consistency of ovarian stimulation (Hughes et al., 2003), an improved control of follicular development, a reduced need for dose adjustment and fewer cancelled cycles (Yeko et al., 2004), and an improved quality of embryos and implantation rate with a trend to higher pregnancy rates, and fewer days of stimulation (Balasch et al., 2004).

In conclusion, the consistent r-hFSH manufacturing process for follitropin alfa FbM has provided a pharmaceutical FSH gonadotrophin preparation that is pure, low in degraded forms of r-hFSH, and with a consistent isoform profile. The introduction of the SE-HPLC assay now allows the precise measurement of r-hFSH content, in follitropin alfa FbM, without the use of an in-vivo rat bioassay. Analytical assessment of commercially available r-hFSH pharmaceutical products has demonstrated that follitropin alfa FbM is the most consistent r-hFSH in terms of protein content.

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