# The cGMP Manufacture of human Macrophage Derived Chemokine for use in a Clinical Trial

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## Abstract

Complex, long peptides for use in Clinical Trials can be manufactured by Solid Phase Peptide Synthesis Technology. The manufacture of h-MDC for clinical trial use is described.

### Almac Solid Phase Peptide Technology

Almac, a service provider to the pharmaceutical industry, has developed Solid Phase Peptide Synthesis (SPPS) methods which unlock the manufacture of long chain, complex peptides. In general, SPPS methodology offers a high degree of control over peptide synthesis and the flexibility to introduce unnatural building blocks and custom labels.

At Almac, over 7000 peptides have been manufactured to date, and the combined experience of these has been captured in Best View Processes for a range of peptide families. This approach enables an experience based Manufacturing Route to be rapidly defined, significantly compressing the timelines for a project, and ensuring a rapid delivery of clinical use material. Almac technologies have been applied to the cGMP manufacture of h-MDC.

### Human-Macrophage Derived Chemokine (h-MDC)

Chemokines are a sub-family of the cytokine family of proteins which have the ability to induce chemotaxis. Chemokines have a high sequence homology, range from 8-10kDa in size, and contain four cysteine residues which form conserved disulfide linkages. h-MDC (CCL22)[1] contains 69 amino acids, and binds specifically to a single chemokine receptor, CCR4. The sequence and a 3-D model of its folded sequence is given in **Figure 1**.


**Figure 1:** Sequence and 3D model of h-MDC

### Challenges in the cGMP Manufacture of h-MDC

h-MDC, as a small protein, presents many synthetic challenges, arising from its length and specific requirement for tertiary structure. Effective characterisation of the product is required, necessitating the development of appropriate analytical methods.

In terms of quality, a high purity product (≥ 95.0% pure) is required, and the product is manufactured according to ICH quality guidelines.

From a commercial perspective, a rapid and cost effective route is necessary. A manufacturing route, and supporting analytical development package was designed to address these challenges.

### h-MDC: The Manufacturing Route

The manufacturing route to h-MDC is summarised in **Figure 2**. Five distinct unit operations were used to manufacture h-MDC as an API. A sterile filtration and lyophilisation step provided the Finished Drug Product as a sterile lyophilsate presented in vials for clinical use.

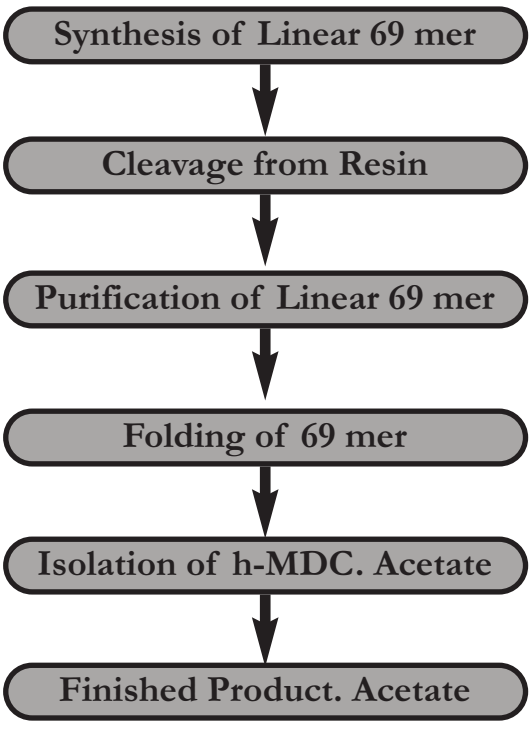


**Figure 2:** Manufacturing route to h-MDC

**The Manufacturing Campaign**

***Synthesis & Cleavage***

Synthesis was carried out by Solid phase peptide assembly on resin. During synthesis, it was essential to achieve a high coupling efficiency at each step, to give an effective product assembly. Active coupling reagents were used, and the process was monitored using UV profiling of Fmoc deprotection. For the synthesis of h-MDC, an average coupling efficiency of > 99% was achieved. Post synthesis, the peptide was cleaved from the resin using a complex scavenger mixture to minimise side reactions. The crude peptide was isolated and an intermediate purification step was performed. Impurities that are closely related to the product were removed. In our cGMP campaign, the main process impurity had a mass of + 16 relative to the desired product, and was identified as the product of methionine oxidation. Deletion sequences and truncates, which are typical process impurities, were also observed.

***Peptide Folding and Isolation***

Peptide folding was the key step in the process to ensure a biologically functional product. The challenge at this stage was to drive the reaction to completion, and overcome side reactions leading to the formation of dimers, misfolds and partially folded products. During cGMP manufacturing a high dilution reaction was applied to achieve the maximum formation of active chemokine. The reaction was monitored by HPLC. Post folding, a purification and isolation step delivered the h-MDC as an acetate salt in ≥ 95.0 % purity.

**Product Characterisation**

h-MDC was characterised using appropriately validated analytical methods. The product was tested under three main areas, namely proof of identity, composition and biological activity. Identity was established by determining the amino acid composition by amino acid analysis, and the peptide was also sequenced by mass spectrometry. Peptide purity, determined using two orthogonal reverse phase HPLC methods, was determined to be > 95.0%. The biological activity of the h-MDC was verified using a product specific bioassay.

***Drug Product - Sterile Filtration***

The h-MDC acetate was filtered under sterile conditions to provide sterile lyophilisate in vials, suitable for use in clinical trials.

**Commercial Challenges**

The drug development process requires progression to clinical trial as rapidly as possible. In the case of h-MDC described above, drug product lyophilisate was manufactured in 6 months.

**References**

1. For an outline in the function of MDC, see Swiss-Prot O00626.

**Author Profiles**

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