

ANALYSIS OF INHALED GLUCOCORTICOSTEROIDS - EPIMERIC BUDESONIDE AND FLUTICASONE PROPIONATE IN HUMAN PLASMA BY LIQUID CHROMATOGRAPHY ATMOSPHERIC PRESSURE CHEMICAL IONIZATION TANDEM MASS SPECTROMETRY (LC/APCI/MS/MS).

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Budesonide and Fluticasone Propionate are two of the leading drugs used in the treatment of asthma and rhinitis, both are topically applied (eg as nasal spray or as an inhalation), with the percentage of the population experiencing asthma or asthma like symptoms these two drugs are excellent aids in their therapy's. The clinical data on the patient samples and the method development will be discussed.

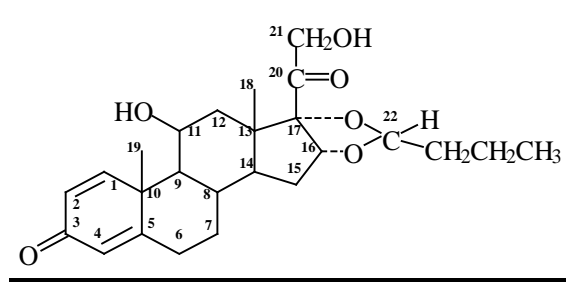
API mass spectrometry is the method of choice for glucocorticosteroids, in particular Atmospheric Pressure Chemical Ionization (APCI) has high sensitivity for this class of compounds, with this we have developed a highly sensitive and selective quantitative assay to determine plasma concentrations from healthy subjects after inhalation of epimeric budesonide (BUD) or fluticasone propionate (FP), respectively. Previous analysis of BUD in thermospray¹ or APCI/LCMS² have been used but had minor problems. Plasma concentrations were quantified in 144 samples using liquid chromatography atmospheric pressure chemical ionization tandem mass spectrometry (LC/APCI/MS/MS). The drugs were isolated from human plasma using a C18 solid phase extraction cartridges BUD was acylated with a mixture of 12.5% acetic anhydride and 12.5% triethylamine in acetonitrile to form the 21- derivatives following the solid -phase extraction. The FP did not have to be derivatised and did not undergo that part of the protocol. Deuterium-labelled budesonide acetate was synthesised and used as the internal standard for both compounds.

The LC method used is able to separate the 22R & 22S epimers of BUD, using 2.1mm ODS column and a simple isocratic elution the sample analysis time is <15min.

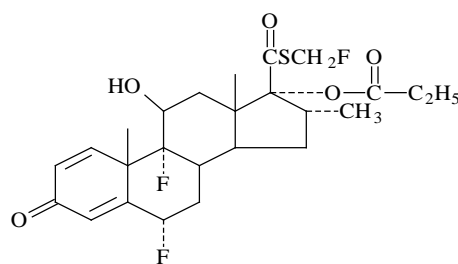
Other assays such as radioimmunoassay (RAI) proved to be good but had suffered poor precision (up to 25% CV). RAI for FP is also subject to nonspecific interference and varying cross -reactivity with drug metabolites and other unrelated compounds, although the antibodies used in the RIA were claimed to be highly specific³.

The assay was linear over the ranges 0.05 -10 ng/ml for BUD and 0.02 - 10 ng/ml for FP, respectively. The interassay and intraassay relative standard deviations were <14.3% in the assay concentration range.

The structures of BUD and FP are shown below.



Budesonide



Fluticasone Propionate

1. Lindberg, C., Blomqvist, A. and Paulson, J. Biol. Mass Spectrom., 21 (1992) 525
2. Li, Y.N., Tattam, B. N., Brown, K.F., Seale, J.P. J.Chromatography. B 683 (1996) 259-268.
3. Makie, A.E., Ventresca, G.P., Fuller, R.W., Bye, A. Br. J. Clin. Pharmacol. 41 (1996) 539 - 542