A DIFFERENTIAL ASSAY FOR CHLORAMPHENICOL AND CHLORAMPHENICOL SUCCINATE BASED UPON A SENSITIVE ASSAY FOR THE PARENT DRUG

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To evaluate prodrugs of an existing drug it is necessary to differentiate between the parent compound and its bioreversible derivative (Notari, 1977). A simple sensitive assay has been developed capable of quantifying chloramphenicol (CAP) and chloramphenicol succinate (CAP-s) at amounts greater than 0.5µg in biological samples.

The optimised assay involves GLC of silylated CAP and tetraphenylethylene as the internal standard (IS) using a flame ionisation detector. Retention times are 9.4 minutes (IS) and 10.8 minutes (CAP).

A rectilinear calibration curve of peak height ratio versus injected amount of CAP results with $r>0.99$ ($n = 12$). The presence of CAP-s and CAP breakdown products do not interfere with this assay.

Extraction of CAP from 2ml aqueous medium into 2ml ethyl acetate is >99% at 25°C provided that the aqueous concentration is <100µg/ml. Subsequent evaporation of the organic solvent (after addition of IS) under a stream of N_2 at 25°C enables dry storage prior to assay.

Although CAP is a neutral molecule whose partition coefficient is pH-independent, CAP-s is an ester of a dibasic acid whose extraction under the conditions described above is <1.5% when the pH of the aqueous phase is greater than or equal to 7.4. Therefore, separation of an aqueous sample at physiological pH into two portions, extraction and assay enables quantification of CAP. Assay of (CAP-s + CAP) in the second portion can be achieved by addition of sufficient aqueous NaOH to attain a pH of 12.1, standing for 30 minutes at 25°C and extracting as before. CAP-s can then be quantified by difference.

The method documented above is based upon alkali catalysed hydrolysis of CAP-s to CAP at pH 12.1 and 25°C. In order to validate the method we have assessed the rate of CAP (GLC) and succinic acid (pH-stat) formation under these conditions. First-order plots of CAP-s concentration versus time were linear ($r>0.99$) for >90% of the process. Repeated determinations of the degradation rate constant showed <5% deviation from the average.

Table 1 documents estimates for the conversion half-life ($t_{0.5}$) determined by both methods. The percentage CAP lost under identical conditions was determined to be <0.5% in a control experiment over 30 minutes

Table 1 Average estimates of the conversion half-life ($t_{0.5}$) and % yield as determined by GLC and pH-stat.

	$t_{0.5}$ (mins)	% yield after 30 minutes
GLC	5.84	>97
pH-stat	6.07	>97

A 30 minute hydrolysis at pH 12.1 has therefore been found to be sufficient to convert >97% CAP-s to CAP at 25°C.

Modifications of this method should enable differential quantification of CAP and CAP esters of any dibasic acid.

Notari, R.E. (1977) in "Design of Biopharmaceutical Properties through Prodrugs and Analogs". Ed. Roche, E.B. pub. Am. Pharm. Assoc.