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The determination of the preservative, chlorocresol, in a pharmaceutical formulation by flow injection analysis*

M.S. BLOOMFIELD† and K.A. PREBBLE

Analytical Development Laboratories, The Wellcome Foundation Limited, Temple Hill, Dartford, Kent DA1 5AH, UK

Abstract: A flow injection analysis (FIA) procedure is described for the determination of chlorocresol in a parenteral pharmaceutical formulation. The product is directly injected into a carrier stream of water and subsequently reacted with a reagent stream of nitrous acid. The resulting brown nitro-derivative is determined spectrophotometrically at 400 nm. The method has been validated and should be applicable to chlorocresol in other pharmaceutical products and to compounds containing a phenolic ring, assuming absence of matrix interference.

Keywords: *Flow injection analysis; chlorocresol; Liebermann reaction; nitrous acid reagent; phenol; Pentostam Injection.*

Introduction

Many pharmaceutical products contain chlorocresol as an antimicrobial preservative and control of the level of this preservative is desirable to assure a satisfactory product. A typical product is Pentostam Injection, an antiparasitic formulation containing sodium stibogluconate for the treatment of leishmaniasis.

A rapid method for the assay of chlorocresol has been developed employing flow injection analysis (FIA). The method is based upon the Liebermann reaction [1], which is an established spot test for aromatic alcohols, particularly phenol. The reaction was applied to chlorocresol, a substituted phenol (4-chloro-3-methylphenol), which reacts with nitrous acid to generate nitro-derivatives which are highly coloured. The undiluted product is injected and dispersed into a carrier stream of water and subsequently reacted with a reagent stream of nitrous acid. The brown derivative is determined spectrophotometrically at 400 nm. Conditions have been established such that the method is linear, accurate and precise over the chlorocresol concentration range 0.05–0.15% w/v in water. The method should be applicable to a wider concentration range by simple adjustment of conditions, assuming obedience of Beer's law is established. No interference is experienced from the large levels of aliphatic alcohol functional groups present from sodium stibogluconate, the active ingredient of Pentostam Injection.

The method is rapid and can be readily automated. The method is suitable for quality assurance to replace the conventional laborious solvent extraction–colorimetric procedure used for this product. The method should be applicable to chlorocresol in other pharmaceutical products and to other compounds containing a phenolic ring, assuming absence of matrix interference can be established.

Experimental

Apparatus

The flow injection manifold is shown schematically in Fig. 1.

Most of the initial development work was carried out using a Gilson 'Minipuls 3' Peristaltic pump equipped with acid resistant tubing, a Magnus Scientific M7100 autosampler equipped with a Rheodyne six-port injection valve, a Cecil Instruments CE 272 spectrophotometric detector equipped with a 10-mm flow cell, and a Bryans Southern Instrument 28000 chart recorder. This equipment was later

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†Author to whom correspondence should be addressed.

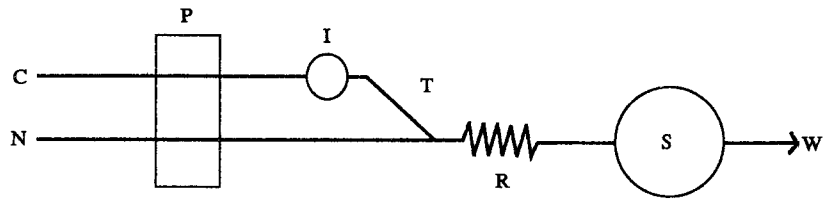

Figure 1
Schematic flow injection manifold. (C) water carrier stream; (N) nitrous acid reagent stream; (P) peristaltic pump; (I) 20 µl injection port; (T) carrier stream tubing, 30-cm long, 0.5-mm i.d.; (R) reaction coil, 200-cm long, 0.5-mm i.d.; (S) spectrophotometer with a detection wavelength of 400 nm, 0.5 a.u.f.s. typical sensitivity; and (W) waste.

replaced with a Burkard 'FIAflo' Flow Injection Analyser System equipped with PTFE six-port valves, a Spectra Physics Focus forward optical scanning detector equipped with a 10-mm flow cell, and a Lloyd Instruments Graphic 1002 chart recorder. PTFE tubing (0.5 mm i.d.) was used for all connections and as reaction tubing.

FIA conditions

The carrier stream was water, pumped at an approximate flow rate of 0.5 ml min^{-1} and the reagent stream was nitrous acid, pumped at an approximate flow rate of 1.8 ml min^{-1}. Nitrous acid reagent was prepared by carefully diluting 200 ml of 98% sulphuric acid to 500 ml in water and adding 25 g of sodium nitrite (both analytical reagent grade, BDH Chemicals, Poole, Dorset, UK) whilst still hot. The reagent was allowed to cool in a stoppered vessel and did not require degassing before use.

The carrier stream tubing was a 30-cm length (measured from the injection point to the confluence with the reagent stream) of 0.5-mm i.d. PTFE tubing, and the reaction coil was a 200-cm length of 0.5-mm i.d. PTFE tubing. The injection volume was 20 µl. A detection wavelength of 400 nm was employed with a sensitivity of 0.5 a.u.f.s.

The undiluted product was the sample solution. An accurately prepared 0.1% w/v solution of chlorocresol (B.P.) in water was employed as the standard solution.

Procedure

The carrier and reagent streams were pumped through the manifold until a stable baseline was obtained. Portions of the standard solution were injected until the repeatability of the measured peak heights was satisfactory. The sample solutions were then injected sequentially, concluding the analysis with further injections of the standard solution. By comparison of the peak heights of sample with the mean of bracketing standard injections the chlororesol content of the product was calculated.

Results and Discussion

Method development

The Liebermann spot test for phenol was applied to chlorocresol, a substituted phenol (4-chloro-3-methylphenol). The resulting derivative was analysed by coupled HPLC–mass spectrometry (Sciex API-III mass spectrometer under ionspray conditions in the negative ionization mode) and NMR (Bruker AC 300 spectrometer), which confirmed that the derivative comprised predominantly two mono- and di-nitro substituted isomers of chlorocresol, with a small amount of coupled dimeric species additionally present. No cleavage of the chlorine functional group in chlorocresol had occurred.

The kinetics of reaction examined as a function of acid strength in an FIA manifold revealed that the reaction time decreased proportionally with increasing acid concentration. A strong acid concentration was required to ensure rapid reaction within the short time-frame of a rapid FIA method. The other components of Pentostam Injection were shown not to interfere.

A single stream system was initially investigated but dispersion and peak shape were improved with a dual stream system employing water as the carrier stream. In this manifold the injection valve was kept separate from the reagent, hence guarding against potential valve damage from the strong acid employed. An experiment to generate nitrous acid *in situ* by combining separate streams of nitrite anion and acid in the dual stream manifold was unsuccessful due to bubble formation from oxides of nitrogen.

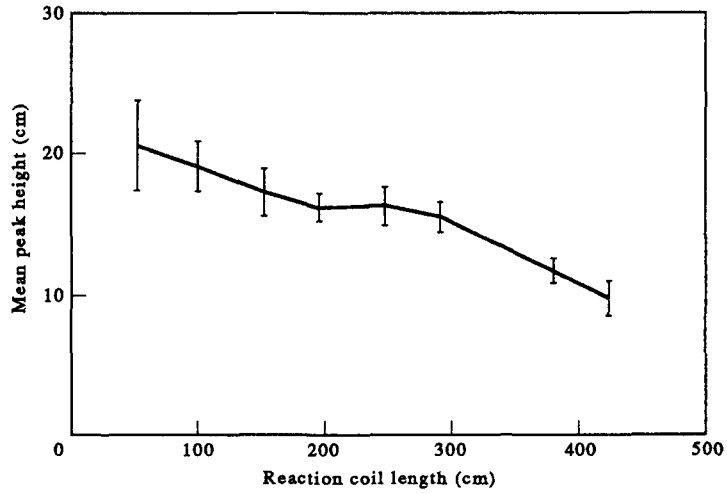

Figure 2
Graph showing peak height as a function of reaction coil length.

Peak height was studied as a function of the reaction coil length at the optimal method flow rates. Results with the confidence intervals ($P = 0.05$) plotted as error bars (eight injections) at each coil length are shown in Fig. 2. Peak height and hence sensitivity were found to increase with reducing reaction coil length but the reproducibility deteriorated. Unsymmetrical peaks were also observed due to inefficient dispersion. An optimum reaction coil length combining good peak shapes and precision with a low reaction time was chosen as 200 cm (0.5 mm i.d.). Typical FIA traces for standard, sample and inert solutions are shown in Fig. 3.

Validation

Validation has been carried out specifically on the application of the method to Pentostam Injection although the method should be applicable to other products.

Linearity of the method. The linearity of the method over the range 0.05–0.15% w/v chlorocresol in water (equivalent to 0–150% of the nominal assay concentration) was confirmed by injecting a series of eight standard solutions at levels of 0, 50, 80, 90, 100, 110, 120 and 150% of the nominal level. A linear response of peak height (as percentage of the 100% standard) vs concentration (percentage of formula content) was obtained with the linear relationship: $y = 0.9946 + 0.47$ (correlation coefficient, 0.9999, standard error of the slope, 0.0060; $n = 8$).

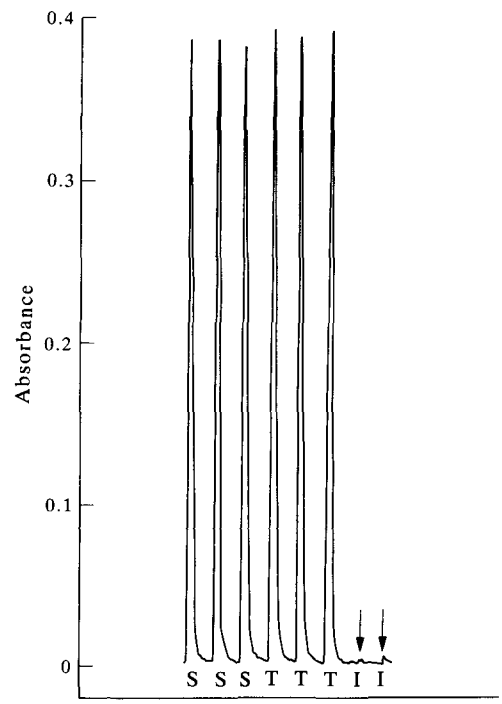

Figure 3
Typical FIA traces for chlorocresolin in a batch of injections. (S) standard solution; (T) sample solution; and (I) inert formulation.

Specificity. An inert formulation containing sodium stibogluconate and glucono-delta-lactone, omitting only the chlorocresol, was examined by the method. A response of 1.0% with respect to the formula content of chlorocresol was obtained. This low level of response was considered acceptable and the method was considered to be specific in the presence of other components in the Pentostam Injection.

Accuracy. The accuracy of the method was determined by recovery experiments. Known quantities of chlorocresol were added to an inert formulation at amounts equivalent to 50, 80, 120 and 150% of the nominal formula content and the mixture assayed by the method. Results obtained were: 97.6, 99.0, 99.8 and 99.7% of the added chlorocresol recovered, respectively. An experiment at the nominal 100% level was performed six times. This gave a mean of 99.5% with a relative standard deviation (RSD) of 1.1%. The 95% confidence interval of the mean value was 99.5 ± 1.1%.

Precision. The repeatability of the method was assessed by carrying out 10 injections of a standard solution (0.1% w/v in water) on a single occasion. An RSD of 1.0% was obtained for the peak height.

For reproducibility, two operators each performed four independent assays on the same batch of product. The mean (as a percentage of the formula content) and RSD of these results were 98.2 and 0.6%, with a 95% confidence interval of the mean of 98.2 ± 0.6.

Influence of detection wavelength. A batch of product was assayed using six different detection wavelengths in the range 380–430 nm (10 nm intervals). Results obtained (as a percentage of the formula content) were 98.6, 98.0, 99.0, 98.5, 97.8 and 97.5 at each wavelength, respectively. It was concluded that small differences in the wavelength of detection did not significantly affect the assay result.

Equivalence of methods. As a further check on the accuracy, the method was applied to three batches of product examined by both FIA and by a classical solvent extraction and colorimetric procedure routinely employed for this purpose. The mean assay results for each batch (as a percentage of the formula content) were 107.9, 109.0 and 98.2 by FIA, compared to 107.7, 108.5 and 98.5, respectively, by the manual procedure. These data demonstrate that the FIA results are equivalent to those obtained by a classical procedure.

Conclusions

Flow injection analysis has been applied to the determination of the preservative chlorocresol in a pharmaceutical product, and the procedure has been validated. The method is rapid and can be readily automated. It should be applicable to chlorocresol in other pharmaceutical products and to other compounds containing a phenolic ring, provided that matrix effects are absent.

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Reference

[1] E.G.C. Clarke, in *Isolation and Identification of Drugs*, Vol. 1. Pharmaceutical Press, London (1969, reprinted 1974).

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