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REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE QUANTIFICATION OF 5-HYDROXYMETHYLFURFURAL AS THE MAJOR DEGRADATION PRODUCT OF GLUCOSE IN INFUSION FLUIDS

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SUMMARY

Analysis of 5-hydroxymethylfurfural (5-HMF) is an important indicator of degradation in glucose infusion fluids. Current pharmacopoeial methods for analysis are insensitive and non-specific. A method is described here, incorporating an internal standard, to give a sensitive, accurate and precise method, suitable for use in the quality control of glucose infusion fluids, and with possible applications for the quantitative determination of 5-HMF in food and drink, bacterial cultures and hydrothermolysed wood.

INTRODUCTION

Sterilisation of infusion fluids by autoclaving is the recommended method, providing that the product can withstand high temperatures. There are, however, some products that are autoclaved although they are known to have some instability, and in these cases their degradation products are controlled by limit tests. The most notable example of this is glucose infusion fluid. The degradation of glucose on heating is known to yield 5-hydroxymethylfurfural (5-HMF) via the formation of unknown intermediates [1–6]. Some of the 5-HMF is then reported to break down to give two acids, 5-hydroxymethylfuroic acid and furan-2,5-dicarboxylic acid[1]. Quantification of the 5-HMF gives an important indication of the extent to which glucose may have been degraded, this being of clinical significance when administering in-

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fusion fluids because of the association of high levels of 5-HMF with thrombophle-
bitis[7,8] and possible interactions of 5-HMF with heparin[9] and other drugs[10].

The current pharmacopoeial monograph for glucose injections includes a limit
test for 5-HMF and related products using a spectrophotometric method based on
the absorption of 5-HMF at 283 nm; this method has been reported to be insensitive
and non-specific[3]. In the present work a sensitive, accurate and precise method is
described, based on reversed-phase high-performance liquid chromatography
(HPLC). An assay for 5-HMF will have applications in various fields of work; assays
have been described for its determination in caramel solutions[11], in hydrothermo-
lysed poplar wood[12], in bacterial cultures[13], as well as in infusion fluids[3]. Our assay
has been applied to the analysis of heat-treated glucose samples, in a series of experi-
ments where 5% (w/v) glucose solutions were heat-treated in a microprocessor-con-
trolled autoclave to examine the effect of various combinations of temperature and
time on the extent of degradation. The qualitative nature of the degradation process
was examined by rapid-scanning photodiode array detection, a technique which will
become of widespread use in the future[14].

EXPERIMENTAL

Materials and methods

The mobile phase was prepared as follows. Sodium dihydrogenphosphate
(AnalaR Grade, BDH, Poole, U.K.) was made up at 0.05 M using glass-distilled
water, the pH adjusted to 5.5 and the solution filtered through a 0.45-μm membrane.
HPLC-grade methanol (Rathburn, Walkerburn, U.K.) was used as the organic mod-
ifier. The mobile phase was degassed under reduced pressure in an ultrasonic bath for
10 min. 5-HMF (Sigma, Poole, U.K.) was used as received. Benzophenone, benz-
aldehyde, vanillin and benzoic acid were obtained from BDH and used as received.
The internal standard, 2-furaldehyde (2-FA) (Aldrich, Gillingham, U.K.) was pre-
pared as a 0.05 M solution in distilled water and introduced into standards and
samples. Glucose Monohydrate BP 5% (w/v) in water was prepared in the Sterile
Production Unit an Ninewells Hospital (Dundee, U.K.) and distributed in 500-ml
glass bottles of the Medical Research Council (MRC) type, fitted with rubber bungs
and aluminium caps.

Equipment

The modular HPLC system consisted of a Gilson 302/802 pump, a Rheodyne
7125 injection valve equipped with a 20-μl loop and a Gilson Holochrome variable-
wavelength detector (the detection wavelength being 283 nm at a sensitivity of 0.05
a.u.f.s.), connected to a Shimadzu C-R1B integrator–printer. The column was 125
mm × 4.6 mm I.D. stainless steel, slurry-packed under pressure with Hypersil ODS
(5 μm) (Shandon Southern Instruments, Runcorn, U.K.). The flow-rate was 2 ml/min
at a pressure of 2000 p.s.i. For diode array detection a Hewlett-Packard HP-1040A
system was used, which consisted of a detector mainframe connected to an HP-85
laboratory microcomputer and an HP82901M dual 5.25-in. flexible disc drive; in
addition a Hewlett-Packard Model 74700 plotter and Model 7222 print-plotter were
used. The autoclave used in degradation work was a Drayton Castle bottled-fluids
steriliser, adapted by CMI (Consolidated Medical Industries, Sunningdale, U.K.) and
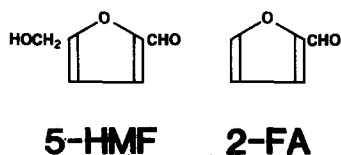
Fig. 1. Structures of 5-hydroxymethylfurfural (5-HMF) and 2-furaldehyde (2-FA).

with microprocessor control using the Apotec 2000 system from IMS (Instrumental and Microelectronic Systems, Rosyth, Fife, U.K.).

Quantitative procedures

Calibration was carried out according to one of two methods — external standardisation using the external standard mode on the Shimadzu integrator and internal standardisation. In the external standardisation mode, full 20-μl loop repetitive injections of a single-concentration 5-HMF standard solution were used for single-point calibration, based on the principle where test samples are "bracketted" by standards injected before and after the test. An internal standard method was also used for comparison. The ratio of the peak area of the 5-HMF to internal standard was used to construct a calibration graph. A number of possible internal standards were explored for 5-HMF quantification. The criteria applied in assessing a suitable candidate were: (1) UV absorption in the region of 283 nm; (2) solubility in the aqueous mobile phase; (3) chemical similarity to 5-HMF; (4) a phase capacity ratio (k') close to that of 5-HMF[15].
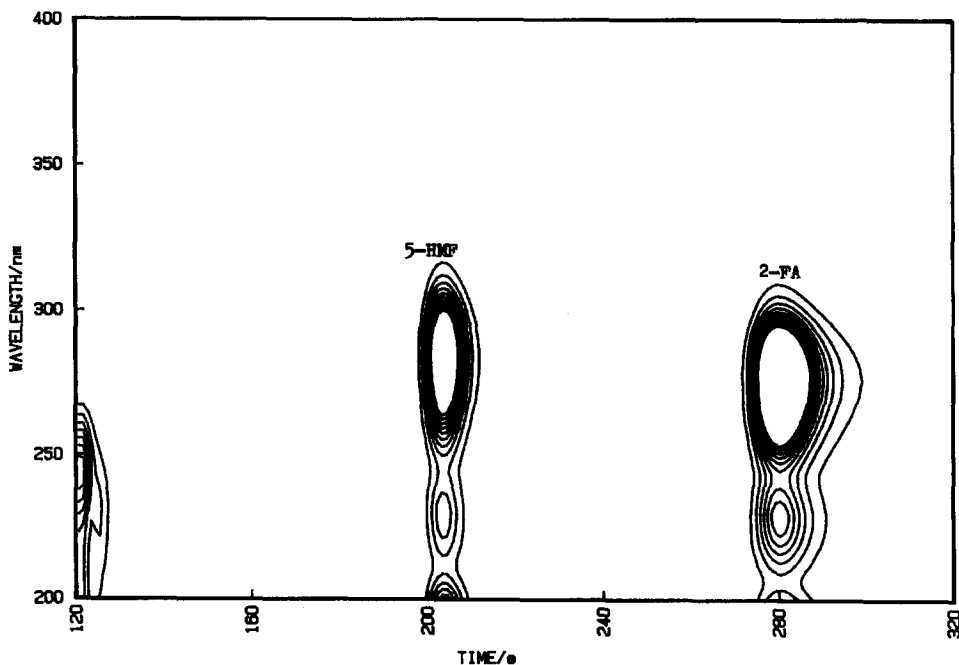

Fig. 2. Contour plot of a degraded glucose sample with internal standard.
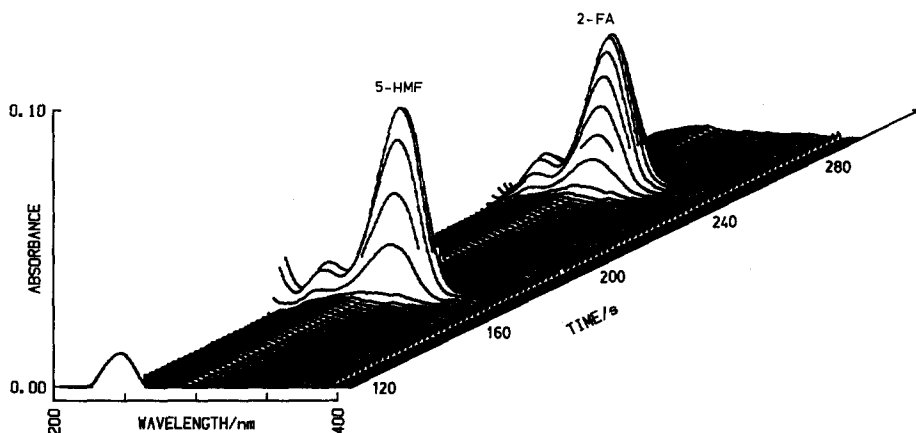
Fig. 3. Three-dimensional plot of a degraded glucose sample with internal standard.

Benzophenone, benzaldehyde, vanillin and benzoic acid were all examined, but apart from their structural dissimilarity to 5-HMF, their phase capacity ratios were too large to be of use. 2-FA was chosen as the internal standard [k' = 3.18; relative standard deviation (R.S.D.) = 0.96, n = 5] since it was well resolved from 5-HMF in the system as finally optimised (k' = 2.05; R.S.D. = 1.0, n = 5) and was chemically similar to 5-HMF (Fig. 1). In addition there have been reports of the simultaneous determination of 5-HMF and 2-FA by HPLC[16] and by fluorimetry[17].

Diode array detection
A diode array detector was used to assess the methodology qualitatively. The rapid acquisition of spectral data at wavelengths from 200 to 400 nm allowed spectra to be displayed at any time segment (*i.e.* for any part of the chromatographic peak). Spectra from the leading and trailing edges and the apex of the peak were normalised for ease of comparison to assess peak purity, thus ensuring that the degradation product being detected at that retention time was 5-HMF by comparison with standards. The resolution of the two peaks was readily viewed by plotting the data matrix as a contour or cartographic plot (Fig. 2), using software developed at The University of Bradford. A more commonly seen display of the data is shown in Fig. 3, which is a three-dimensional or pseudoisometric plot.

RESULTS AND DISCUSSION

Eluent optimisation
Inspection of Fig. 4 indicates that at pH 5.5 with an organic modifier concentration of 15% (v/v), a satisfactory k' for 5-HMF is observed (2.05; R.S.D. = 1.0, n = 5) with good column performance (N = 30 000 plates/m). Resolution from the internal standard is excellent (Fig. 5). The effect of pH on separation was studied, and it was found that the k' values for 5-HMF and 2-FA were constant and that high column performances were maintained (Fig. 6). The optimum flow-rate for high sample
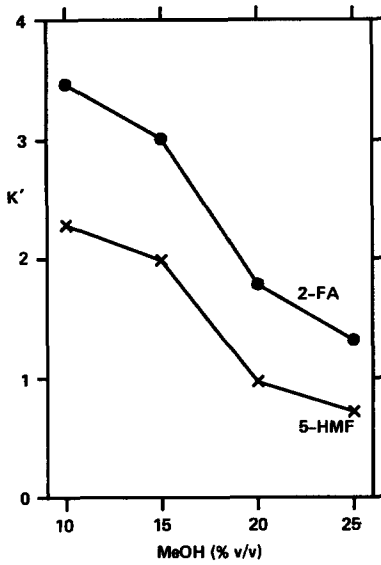
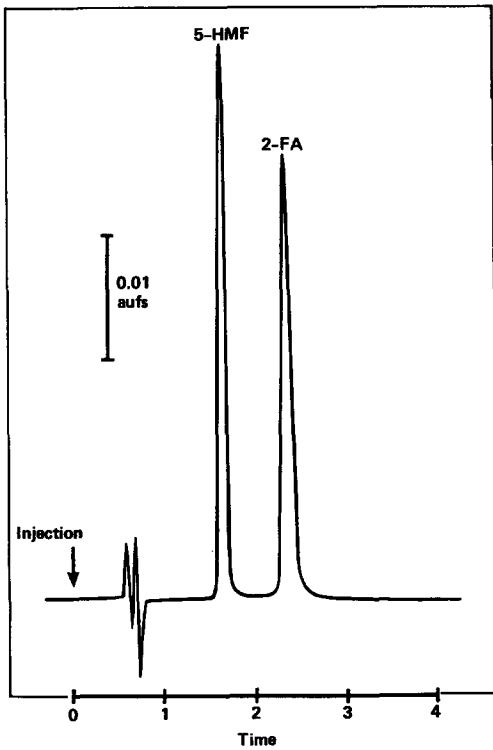Fig. 4. Plot of k' against methanol concentration in mobile phase at pH 5.5 and a flow-rate of 2.0 ml/min.

Fig. 5. Chromatogram of 5 μg/ml 5-hydroxymethylfurfural (5-HMF) and 2-furaldehyde (2-FA) under optimised conditions (see text).
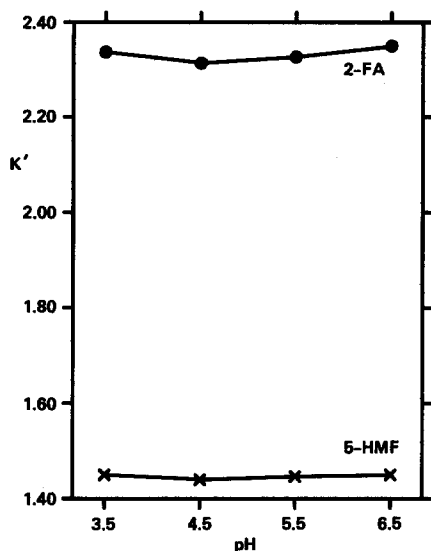
Fig. 6. Plot of k' against pH of buffer portion of mobile phase.

throughput coupled with good resolution of 5-HMF and 2-FA was found to be 2.0 ml/min.

Quantitative comparison of external and internal standard methods

External standard calibration gave a linear response for peak-area ratio against 5-HMF concentration at 0.05 a.u.f.s. over the range 1 – 5 μg/ml, the parameters derived from least-squares regression being $y = 35\,500x - 1540$; $n = 6$; $r = 0.993$. Internal standard calibration displayed a linear regression for peak-area ratio against 5-HMF concentration over the same concentration range at 0.05 a.u.f.s.: $y = 1.90x + 0.024$; $n = 6$; $r = 0.998$.

The R.S.D. of replicate injections for the external standard method at 1 μg/ml was 4.32% ($n = 5$) and for the internal standard method at this concentration was 1.30% ($n = 5$). The limit of detection of the internal standard method at a signal-to-noise ratio of 2 was 3.2 ng on-column of 5-HMF (R.S.D. = 7.8%; $n = 5$). Linearity of the two methods was comparable, but the internal standard method gave superior reproducibility and was therefore the method of choice.

Although there have been some reports that 2-FA may be a degradation product[6,18], these have not been confirmed in the present work. No interference with the internal standard was found in heated glucose solutions under the pharmaceutical sterilisation conditions employed. The method outlined has been found to be suitable for quantitative analysis of 5-HMF produced during heat sterilisation of glucose parenterals.

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