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Difference spectrophotometric assay of 5-hydroxymethylfurfuraldehyde in hydrolysed pharmaceutical syrups — II. Isoniazid reagent

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Abstract: A rapid difference spectrophotometric assay of 5-hydroxymethylfurfuraldehyde (5-HMF) in certain degraded syrups is described. The method involves the measurement of the difference absorbance at 340 nm of the isonicotinoyl hydrazone of 5-HMF, formed at room temperature in an acidic solution of isoniazid, relative to an equimolar solution of 5-HMF, which has been reduced by sodium borohydride, and isoniazid reagent. The procedure is accurate, precise and selective for 5-HMF in the syrups examined. The limits of detection and determination are 0.91 μg ml^{-1} and 12.4 μg ml^{-1}, respectively. The method has been applied to codeine linctus, paracetamol elixir (paediatric) opiate squill linctus, ipecacuanha and squill linctus, Phensedyl Linctus and invert syrup.

Keywords: *5-Hydroxymethylfurfuraldehyde; difference spectrophotometry; pharmaceutical syrups; codeine linctus; paracetamol elixir (paediatric); opiate squill linctus; ipecacuanha and squill linctus; Phensedyl linctus; invert syrup.*

Introduction

Part I [1] describes the assay of 5-hydroxymethylfurfuraldehyde (5-HMF) in certain syrup formulations. The method is based on the measurement of the absorbance at 283 nm of a solution of the syrup relative to that of an equimolar solution of the syrup in which the 5-HMF is reduced by the addition of sodium borohydride. Application of the procedure is limited to those syrups which on dilution and then reduction with sodium borohydride give a solution that has an absorbance of less than 1 at 283 nm.

This paper describes an alternative difference spectrophotometric procedure in which the absorbance of the 5-HMF treated with isoniazid reagent to form a derivative which has a wavelength of maximum absorption at around 340 nm is measured relative to that of an equimolar solution of reduced 5-HMF similarly treated with isoniazid reagent.

Experimental

Reagents

5-Hydroxymethylfurfuraldehyde, D-glucose, D-fructose, sucrose, sodium borohydride and sodium borohydride reagent were as described previously [1].

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Isoniazid (isonicotinic acid hydrazide) (BDH Chemicals, Poole, UK). Isoniazid reagent was prepared by dissolving 1 g of isoniazid in 17.6 ml of hydrochloric acid (S.G. 1.18) and diluting to 100 ml with water. The reagent is stable for at least 1 month.

Equipment

Absorption and difference absorption spectra were recorded as described earlier [1]. Difference absorbances of standard, sample and blank solutions at 340 nm were read from the digital display under non-scanning conditions. High-performance liquid chromatography was carried out as described earlier [1].

Procedure

Standard solutions. A standard solution of 5-HMF was prepared by dissolving approximately 80 mg, accurately weighed, in 5 ml of ethanol and diluting to 1 l with water. A 5-ml aliquot was transferred to two 50-ml volumetric flasks. Sodium borohydride reagent (2 ml) was added to one flask and when the evolution of hydrogen had ceased (after about 5 min), isoniazid reagent (5 ml) was added to each flask. The contents of both flasks were diluted to 50 ml with water and allowed to stand for 20 min. The difference absorbance at 340 nm (ΔA_{340}) of the hydrazone derivative of 5-HMF was measured relative to that of the solution treated with sodium borohydride in the reference cell.

Sample solutions. The weight per ml of the syrup was determined by weighing 10 ml of the syrup contained in a 10-ml volumetric flask. A quantity of the syrup equivalent to about 5 ml was weighed accurately into a 50-ml beaker and 15 ml of water were added. Alternatively for low viscosity samples, a "to contain" pipette was used to transfer 5 ml of the syrup to the beaker and the pipette was rinsed with 3 × 5 ml of water. The pH of the solution was measured using a pH-meter and, if necessary, it was adjusted to pH 8 by adding drops of 0.1 or 1 M sodium hydroxide. The solution was then transferred with rinsing to a 25-ml volumetric flask and diluted to volume with water. Equal volumes of the solution, up to 10 ml containing up to 400 μg of 5-HMF, depending on its concentration in the sample, were transferred to two 50-ml volumetric flasks and the procedure was continued as described above for the standard solutions from the words "sodium borohydride reagent (2 ml) was added . . .".

The concentration of 5-HMF in the sample solutions, and hence in the syrup sample was calculated from the proportional relationship that exists between the difference absorbance at 340 nm and the concentration of 5-HMF.

Results and Discussion

Isoniazid is one of a number of widely used hydrazide reagents that form coloured hydrazone condensation products with substances containing a carbonyl group. Condensation requires an acidic medium to allow the reaction to proceed quantitatively to completion.

The absorption spectrum of the isonicotinoyl hydrazone of 5-HMF, prepared by treating a solution of 5-HMF with a reagent containing isoniazid and hydrochloric acid, recorded against water is shown in Fig. 1a. Also shown in Fig. 1a is the absorption spectrum of a solution containing 5-HMF reduced with sodium borohydride, isoniazid and hydrochloric acid and that of a solution of isoniazid in hydrochloric acid, both

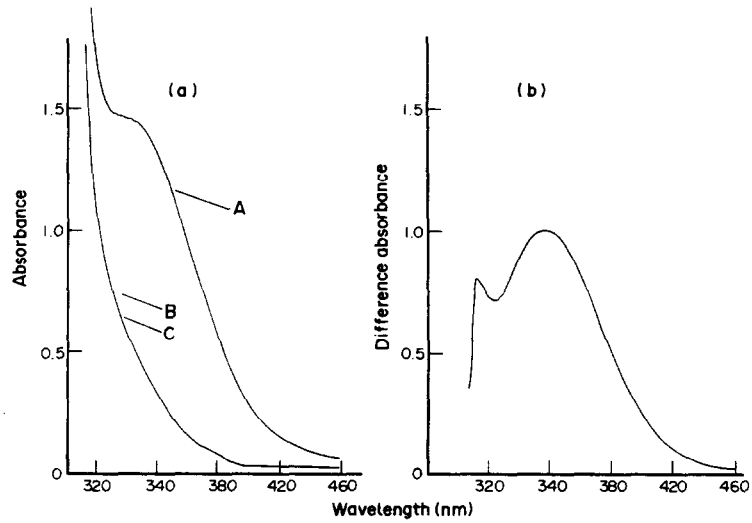

Figure 1
(a) The UV absorption spectrum of (A) 5-HMF (10 μg ml^{-1}) in the presence of isoniazid reagent (B) reduced 5-HMF (10 μg ml^{-1}) in the presence of isoniazid reagent and (C) isoniazid reagent. Spectra (B) and (C) are identical. (b) The difference absorption spectrum of solution (A) relative to solution (B).

recorded against water. The solution of the hydrazone has a λ_{max} around 340 nm and is pale yellow in colour owing to the tail of the absorption band extending into the visible region of the spectrum. The spectra of reduced 5-HMF treated with the isoniazid reagent and of the isoniazid reagent itself are identical and show end absorption at 340 nm. The difference absorption spectrum (Fig. 1b) of the solution of 5-HMF treated with isoniazid reagent recorded against an equimolar solution of reduced 5-HMF containing isoniazid reagent in the reference cell shows a maximum at 340 nm. Noise in the difference spectrum below 300 nm is due to the intense absorption of light by the isoniazid reagent in both the sample and reference cells.

In order to establish the optimum composition of the isoniazid reagent for the difference spectrophotometric assay of 5-HMF, the absorbance at 340 nm of solutions of 5-HMF, after reaction for 15 min at room temperature with different reagents in which the concentrations of isoniazid and hydrochloric acid were varied independently, were measured relative to a blank solution containing the appropriate concentrations of isoniazid and hydrochloric acid. The absorbances of these blank solutions were also measured at 340 nm relative to water. The results in Fig. 2a show that maximum formation of the hydrazone derivative is obtained with a low concentration of acid and a high concentration of isoniazid. However, reagents with a low concentration of acid and a high concentration of isoniazid give blank solutions with very high absorbances at 340 nm (Fig. 2b) which reduce the precision and sensitivity of the difference absorbance of 5-HMF. As a compromise, a reagent that on dilution gives a final concentration of 0.1% m/v of isoniazid and 0.2 M hydrochloric acid was selected.

The time required for maximum formation of the derivative was determined by measuring the absorbance at 340 nm of a solution of 5-HMF (10 μg ml^{-1}) every 5 min after the addition of the reagent. Maximum formation of the derivative occurred within 10 min and the absorbance was stable for at least 2 h.

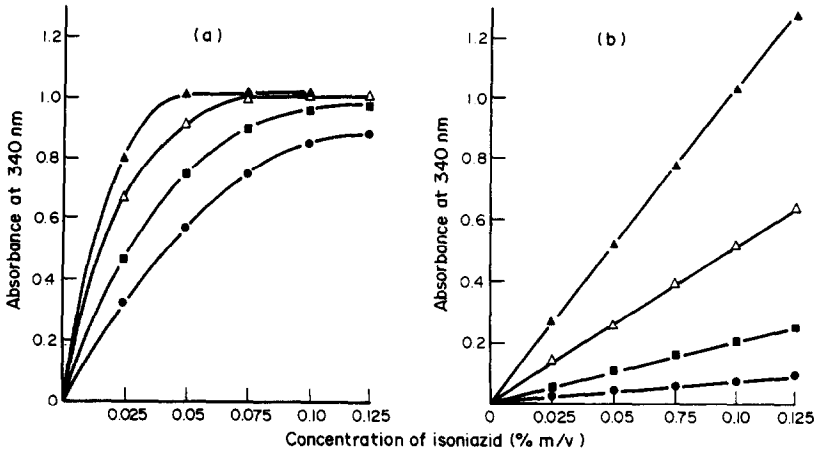

Figure 2
The optimisation of the composition of the isoniazid reagent (a) The net absorbance at 340 nm of 5-HMF in reagents containing different concentrations of isoniazid and hydrochloric acid. (b) The absorbance at 340 nm of the blank reagents containing different concentrations of isoniazid and hydrochloric acid relative to water. —▲—▲— 0.05 M HCl; —△—△— 0.1 M HCl; —■—■— 0.2 M HCl; —●—●— 0.5 M HCl.

Validation

Beer's Law graphs showed that a proportional relationship exists between the concentration of 5-HMF and the ΔA_{340} of solutions of 5-HMF treated with isoniazid reagent relative to an equimolar solution of reduced 5-HMF treated with isoniazid reagent. The regression equations for calibration series of dilutions containing 0, 2, 4, 6, 8 and 10 μg ml^{-1} of 5-HMF in the presence and absence of sucrose 5% m/v, glucose 5% m/v and laevulose 5% m/v are shown in Table 1. The sugars at a concentration of 5% m/v were found not to interfere with the ΔA_{340} of 5-HMF. To assess further the specificity of the method for 5-HMF in syrups containing sucrose, glucose or laevulose, the ΔA_{340} of a series of solutions containing 5-HMF (10 μg ml^{-1}) and increasing concentrations of the sugars (0, 1, 2, 3, 4, 5 and 6% m/v) was measured. The ΔA_{340} of all the solutions containing sugars fell within the range 98.8–100.9% of that of 5-HMF alone confirming that the ΔA_{340} is independent of the concentrations of the sugars up to 6% m/v.

It was observed during these experiments that the absorbance at 340 nm of the reference solution containing reduced 5-HMF, dextrose and isoniazid reagent increased slowly with time. The effect was also observed in a solution of dextrose and isoniazid reagent and in one of sodium borohydride, dextrose and isoniazid reagent. Small differences between the absorbance of these two solutions were found to occur in the first 15 min after the addition of the isoniazid reagent but these differences reduce to a negligible value after this time. Consequently, the time of measurement of the ΔA_{340} of the syrups containing dextrose was selected to be 20 min.

Statistical evaluation of replicate results obtained with Codeine Linctus (Table 2, Sample 3) showed that the mean concentration of 5-HMF and the relative standard deviation were 60.1 μg ml^{-1} and 1.32% respectively. The limits of detection and determination calculated statistically as described earlier [1] were 0.91 μg ml^{-1} and 12.4 μg ml^{-1} respectively. These limits were of a similar magnitude to those calculated for the earlier method involving the reduction of 5-HMF and measurement of ΔA_{283} [1].

Table 1
Calibration data

Composition of solutions	Regression equation*	r†
5-HMF	$y = 0.1006x - 0.001$	0.9999
5-HMF + glucose (5%)	$y = 0.1013x + 0.006$	0.9998
5-HMF + laevulose (5%)	$y = 0.1010x + 0.006$	0.9999
5-HMF + sucrose (5%)	$y = 0.1001x + 0.006$	0.9998

*$y = \Delta A_{340}$, x = concentration of 5-HMF in μg ml^{-1}.
†r = correlation coefficient ($N = 6$).

Table 2
Assay results

Sample	Manufacturer*	Age (years)	Initial pH†	Concentration of 5-HMF (μg ml^{-1})	
				This method	HPLC [2]
1. Opiate Squill Linctus	A	7/12	3.4	69.4	72.6
2. Ipecacuanha and Squill Linctus (paed)	B	1 4/12	2.9	14.1	15.5
3. Codeine Linctus	C	11/12	2.9	60.1	60.0
4. Codeine Linctus	D	11	2.6	403.9	398.8
5. Paracetamol Elixir (paediatric)	E	11	3.9	208.4	205.9
6. Paracetamol Elixir (paediatric)	B	1 2/12	3.0	74.4	72.4
7. Phensedyl Linctus	F	10 7/12	2.4	202.1	204.0
8. Invert Syrup	G	5	3.5	258.3‡	262.0
9. Invert Syrup	G	3 1/12	4.2	204.4‡	201.8
10. Invert Syrup	G	2 9/12	4.4	120.7‡	118.4
11. Invert Syrup	G	9 9/12	4.1	220.8‡	222.9

*A = CP Pharmaceuticals; B = William Ransom; C = Berk Pharmaceuticals; D = Cooperative Wholesale Society Ltd.; E = Onward Pharmaceutical Services Ltd.; F = May and Baker Ltd.; G = Thornton and Ross Ltd.
†pH of syrup diluted 1 + 3 with water.
‡Samples 8, 9, 10 and 11 were also assayed by the earlier difference spectrophotometric assay. For a comparison of the results refer to Table 3 of [1] (Samples 3, 4, 5 and 6 respectively).

A comparison of the sensitivities of the two procedures, in terms of the slopes of the calibration graphs (Table 1; cf. Table 1 of [1]) and the $A^{1\%}_{1\,cm}$ values calculated using the values obtained for the slopes, showed that this procedure, in which the $A^{1\%}_{1\,cm}$ value of 5-HMF is 1006, is only slightly less sensitive than the earlier procedure in which the $A^{1\%}_{1\,cm}$ value was found to be 1296.

Applications and assay results

The present method extends the range of syrups that can be assayed spectrophotometrically for 5-HMF to include some syrups that cannot be assayed by the measurement of difference absorbance at 283 nm because of the intense absorption by their drug or other components at 283 nm [1].

The results of the assay of 5-HMF in syrups to which the present method has been applied are given in Table 2. For comparison, the results obtained by using a variation [1]

of the high-performance liquid chromatographic procedure of Durham *et al.* [2] are also reported.

The results in Table 2 indicate that good agreement was obtained between the assay values obtained by using the difference spectrophotometric procedure and those obtained by using the HPLC procedure, and confirm the accuracy and selectivity of the proposed method. Also, the results for four samples of Invert Syrup were similar to those obtained by using the earlier difference spectrophotometric procedure [1], showing the good agreement of the difference spectrophotometric procedures for syrups to which both methods are applicable.

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References

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[2] D. G. Durham, G. T. Hung and R. B. Taylor, *Int. J. Pharm.* **12**, 31–40 (1982).