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Quantitation of 5-hydroxymethylfurfural (5-HMF) and related substances in dextrose injections containing drugs and bisulfite

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Abstract

The spectrophotometric test for 5-hydroxymethylfurfural and related substances, which is included in multiple compendial monographs for dextrose-containing injections, was modified to eliminate interference of bisulfite anion. Experimental formulations containing heparin or dopamine hydrochloride were used in this study. The modified assay was shown to be accurate, precise and rugged. With an appropriate standard, the method can be used to measure 5-HMF in solutions with an approximate quantitation limit of 0.06 ppm.

Keywords: Bisulfite; Dopamine; Heparin; 5-Hydroxymethylfurfural; 5-Hydroxymethylfuraldehyde; Spectrophotometry

1. Introduction

Aqueous dextrose solutions upon heating undergo sequential elimination of water molecules resulting in the formation of 5-hydroxymethylfuraldehyde (5-hydroxymethylfurfural, 5-HMF) and its related substances (Fig. 1). Several papers describing the mechanism and kinetics of this reaction have been published [1–6], but no consensus has been reached about the structures of 'related substances'. Since many injectable pharmaceutical products are formulated with dextrose, control of its potential degradation is important, especially after terminal sterilization.

A test to limit impurities related to 5-HMF was proposed in the September–October 1977 issue of Pharmacopeial Forum [7], and the first test for 5-hydroxymethylfurfural and related substances appeared in a Dextrose Injection monograph in the Fourth Supplement to USP XIX. It became official on 1 May 1978. The test procedure requires diluting an aliquot of dextrose injection solution with water, such that 1.0 g of dextrose monohydrate is contained in 250.0 ml of solution. The absorbance of the diluted solution is measured at 284 nm, and the upper limit is 0.25 absorbance unit, which corresponds to a concentration of approximately 1.9 ppm of 5-HMF.

In addition to Dextrose Injection, the test is included in many USP 23 monographs for injec-

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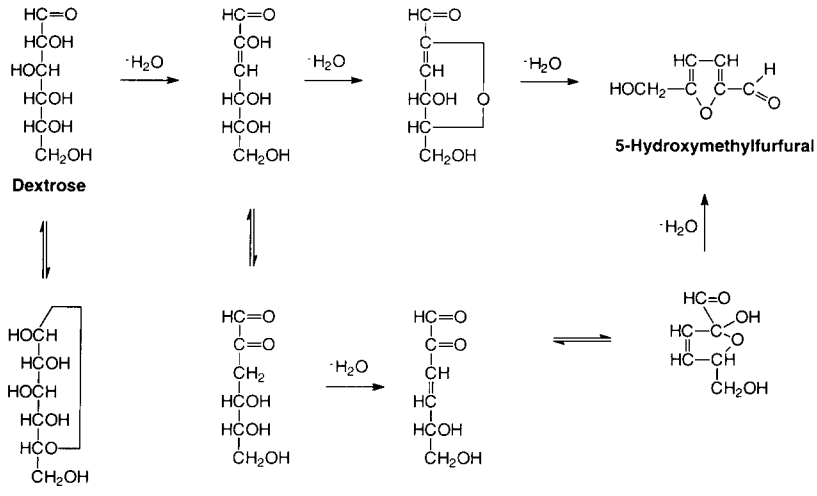


Fig. 1. Possible mechanism of 5-HMF formation by dehydration of dextrose.

tions with dextrose and electrolytes (such as sodium [8] or potassium chloride [9]), Ringer's and lactated Ringer's [10], alcohol [11] and theophylline [12]. In these monographs, the test specifies a dilution of 1.0 g of dextrose monohydrate in 500.0 ml of solution, and the 0.25 absorbance unit limit applies to all products. The USP also contains multiple examples of monographs for dextrose-containing drug injections that do not include the test [13–16]. The amount of 5-HMF found in parenteral solutions was determined not to pose a toxicological problem [17].

Since the compendial test is based on the bulk absorbance of a sample, the presence of any substance absorbing at 284 nm interferes with the determination of 5-HMF and related substances. This is a common occurrence with dextrose injections containing pre-mixed drugs. Reports have been published describing chromatographic separations of 5-HMF from drug containing matrices [18,19]. In an earlier study performed in this laboratory [20], a procedure was developed for removing an interfering drug, theophylline, from Theophylline in Dextrose Injection on a cation-exchange column prior to the spectrophotometric test. This approach was later adopted in a revised USP monograph [12].

Another source of interference is the presence of bisulfite, which is used in some injections as antioxidant. Such solutions exhibit reduced UV absorbance at 284 nm (Fig. 2) and therefore underestimate the actual level of 5-HMF and related substances. The spectral changes are likely caused by an interaction of bisulfite anion with the aldehyde group of 5-HMF (Fig. 3). This paper describes a validated procedure for eliminating this interference, which is accomplished by the addition of sodium hydroxide to the sample. The alkaline diluent, 0.01 N sodium hydroxide, causes a conversion of bisulfite ion into sulfite ($\log K = 6.79$ [21]), reversing the aldehyde–bisulfite adduct formation, and allowing spectrophotometric determination of 5-HMF.

2. Experimental

2.1. Materials

5-HMF reference material was purchased from Aldrich Chemical Company (Milwaukee, WI), dopamine hydrochloride from BASF Fine Chemicals (Pirsippany, NJ), haparin from Diosynth Inc. (Chicago, IL), dextrose, sodium bisulfite and dibasic sodium phosphate from Mallinckrodt Inc. (Paris, KY), sodium hydroxide from Ricca Chem-

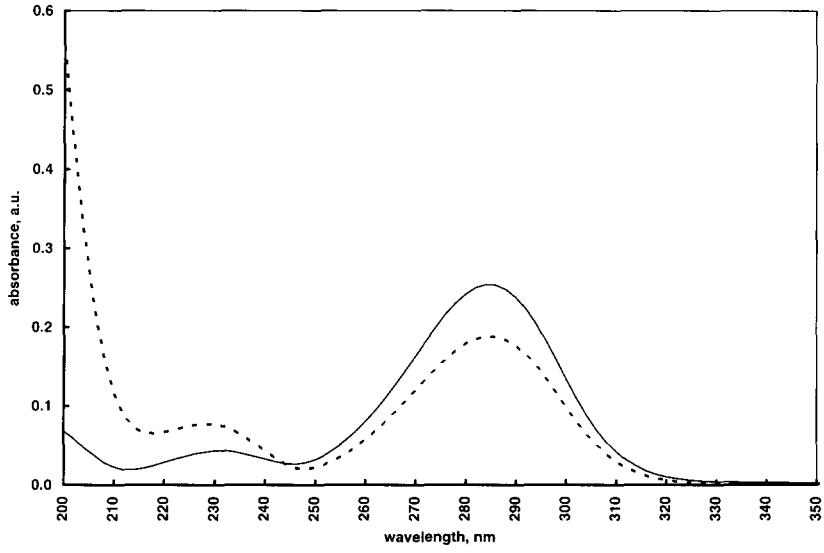


Fig. 2. UV spectra of 5-HMF in water, approximately 1.9 ppm (solid line), and of the same solution with approximately 20 ppm of sodium bisulfite added (dashed line).

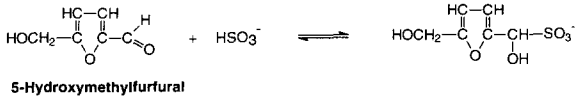
ical Company (Arlington, TX) and citric acid from Fisher Scientific Company (Fair Lawn, NJ). Water processed through a Sybron/Barnstead NANOpure II system (Boston, MA) was used in all the experiments.

Experimental herparin formulations contained approximately 100 units ml^{-1} of heparin in a phosphate–citrate (pH 6) buffer (approximately 7 mM phosphate–3 mM citrate), 5% dextrose and 0.02% sodium bisulfite. Dopamine formulations contained approximately 3.2 mg ml^{-1} dopamine hydrochloride, 5% dextrose and 0.05% sodium bisulfite. The test solutions were spiked with various amounts of 5-HMF.

2.2. Assay procedures

2.2.1. Heparin

A 2 ml volume of heparin–dextrose solution was pipetted into a 50 ml volumetric flask, 5 ml of 0.1 N sodium hydroxide were added, and the flask contents were diluted to volume with water. The absorbance of this solution was determined in a 1 cm cell at 284 nm, with 0.01 N sodium hydroxide as a blank.



5-Hydroxymethylfurfural

Fig. 3. Adduct formation of 5-HMF and bisulfite ion in aqueous solution.

2.2.2. Dopamine

A 2 ml volume of dopamine hydrochloride–dextrose solution was pipetted into a water-washed disposable cation-exchange column (Poly-Prep® Prefilled Chromatography Columns with AG50W-X8 (H^+) packing, purchased from Bio-Rad Laboratories, Richmond, CA). The effluent was collected in a 50 ml volumetric flask. The column was washed with 25 ml of water and the washings were collected in the same volumetric flask. The flask contents were diluted to volume with 0.022 N sodium hydroxide (approximately 23 ml) and the absorbance of this solution was determined in a 1 cm cell at 284 nm with the 0.01 N sodium hydroxide diluent as a blank. Two types of spectrophotometers were used: a photodiode-array spectrophotometer (Hewlett-Packard Model 8452A) and a double-beam spectrophotometer (Hitachi Model U3110).

3. Results

3.1. Evaluation of method performance

The limit of 0.25 absorbance unit listed in the USP test for 5-HMF and related substances corresponds to approximately 1.9 ppm of 5-HMF in a diluted test solution, which represents approximately 47.5 ppm of 5-HMF in experimental heparin and dopamine formulations. All experiments were designed to assess method performance at or near this 5-HMF level, henceforth referred to as the nominal level. The results of experiments were expressed as percentage recovery of 5-HMF, which was calculated by dividing the measured concentration of 5-HMF by its theoretical content and multiplying by 100. The 5-HMF concentrations were obtained from the linear regression of absorbance readings of 5-HMF standards prepared in water versus their concentration. The standards were prepared by volumetric dilutions of the 5-HMF stock solution used to spike the experimental formulations. When appropriate, relative standard deviation (RSD) values and the number of replicates (n) are listed.

3.2. Effectiveness of dopamine removal by a cation-exchange column

Since dopamine absorbs at 284 nm, the effectiveness of its removal could be assessed by analyzing the experimental formulation as described above for dopamine, and in the same way but omitting the cation-exchange column cleanup step. The recovery values at the nominal 5-HMF level were 100.5% with a cleanup procedure (RSD = 0.42%, $n = 4$) and over 700% for the formulation diluted without the column cleanup. The excess absorbance was due to the presence of dopamine in the test solution. In a separate experiment, the cation-exchange column cleanup was shown to be effective at up to 7.2 mg ml^{-1} dopamine hydrochloride, but no attempt was made to determine the upper limit of the column capacity.

3.3. Effect of sample pH and amount of base added

Since the elimination of bisulfite interference requires raising the solution pH, the effect of experimental formulation pH on the 5HMF recovery was investigated. the diluted sample, prepared for UV analysis as described above, contains 0.01 N sodium hydroxide. In this experiment the amount of base was varied from 0.004 to 0.02 N, to verify the ruggedness of the assay with respect to the concentration of the base diluent. Dilution with water was included to demonstrate the extent of interference. The results obtained are listed in Table 1. Between 96% and 104% of the theoretical amount of 5-HMF was recovered from the heparin formulations and between 98% and 101% from the dopamine formulations. The precision of quantitation (RSD, $n = 4$) was 2% or better. A heparin test preparation diluted in water showed less than 90% recovery and the dopamine formulation, containing more bisulfite, less than 80%.

3.4. Stability of absorbance readings

The stability of 5-HMF in diluted test preparations was assessed by performing UV analyses on the same samples immediately after preparation and after 1 and 3 h. The recovery was essentially quantitative at all time points, although a slight downward trend was observed. the greatest recovery difference between the immediate and 4 h readings did not exceed 3%.

3.5. Test sensitivity, linearity and limit of quantitation

The test sensitivity to 5-HMF was approximately 0.13 absorbance units ppm^{-1}. The response linearity in the range 75–125% of the nominal concentration was excellent with a correlation coefficient greater than 0.999. The limit of quantitation depends on the spectrophotometer used. On a photodiode-array spectrophotometer, the reading precision for $n = 3$ reached 10% (RSD) at approximately 0.01 absorbance units.

Table 1
Effect of formulation pH and amount of added NaOH on 5-HMF recovery

Formulation	pH	Final NaOH concentration (N)	Mean 5-HMF recovery (%)	RSD (%) ($n = 4$)
Heparin	4.9	0.004	97.2	2.1
	4.9	0.01	98.2	0.1
	4.9	0.02	96.3	0.1
	5.8	0.004	102.9	0.2
	5.8	0.01	97.2	0.5
	5.8	0.02	103.0	1.4
	5.8	Water	88.9	1.0
	6.0	0.004	100.2	1.6
	6.0	0.01	97.0	0.4
	6.0	0.02	104.3	0.1
Dopamine	3.2	0.004	101.1	0.8
	3.2	0.01	99.4	0.5
	3.2	0.02	99.0	0.7
	3.6	0.004	100.7	0.4
	3.6	0.01	100.5	0.4
	3.6	0.02	98.4	0.9
	3.6	Water	78.2	1.7
	4.0	0.004	100.9	1.7
	4.0	0.01	99.9	1.0
	4.0	0.02	98.7	0.9

The dual-beam spectrophotometer performed considerably better, still generating precise readings at 0.003 absorbance units (RSD < 2%). The response linearity between 0.4 and 0.06 ppm was excellent with a correlation coefficient of 0.9999.

4. Conclusion

Many dextrose-containing injections require control of the 5-HMF content, which is formed from dextrose during terminal sterilization. The USP spectrophotometric test for 5-HMF and related substances, adapted to the analysis of bisulfite-containing solutions, was shown to be accurate, precise, sensitive, linear and rugged. Its simplicity makes it ideal for use in a quality control environment. The quantitative determination of 5-HMF content in the sample is possible if an appropriate standard is used.

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