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Quantification of 5-HMF and dextrose in commercial aqueous dextrose solutions

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Abstract

5-Hydroxymethylfurfural (5-HMF) is the major thermal decomposition product formed in aqueous dextrose solutions during sterilization and upon storage. Current compendial tests employ a spectrophotometric assay for 5-HMF and a separate assay based on optical rotation for dextrose. Efforts herein focused on identification of approaches to simultaneously quantify both 5-HMF and dextrose in aqueous solutions. Initial studies employed anion exchange chromatography with pulsed amperometric detection (PAD). These studies showed that the low anion exchange column loading capacity and concentration mismatch of dextrose and 5-HMF in typical commercial solutions makes simultaneous quantitation impractical at a single sample concentration. However, mixed modes of detection for each analyte using refractive index (RI) detection for dextrose and UV detection for 5-HMF proved viable for simultaneous quantitation. The performance of both approaches was evaluated and extremely high sensitivity for 5-HMF demonstrated (30–50 ppb). The formation of 5-HMF upon the forced thermal decomposition of dextrose was monitored and compared with results from the USP spectrophotometric assay.

Keywords: 5-Hydroxymethylfurfural; Dextrose; Assay; Anion-exchange chromatography; Pulsed amperometric detection; Ion-exchange chromatography; Refractive index

1. Introduction

The thermal degradation of aqueous dextrose solutions has been extensively studied and 5-hydroxymethylfurfural (5-HMF) firmly established as the principal decomposition product in acidic solution [1–16]. Both the kinetics and mechanism of dextrose conversion to 5-HMF have been explored in detail, and color and particulate matter formation in pharmaceutical products have been related to further decomposition products of 5-HMF [1–6]. A decrease in solution pH has also been reported in thermally stressed dextrose solutions and a series of acids have been identified that are believed to account for the increased acidity [7]. Additional studies have linked the decomposition of dextrose during steam sterilization to processing conditions such as cycle duration and temperature [8–12].

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The well-documented thermal instability of dextrose solutions is a particularly important consideration for use of these solutions as intravenous infusion fluids, as the solutions are subject to decomposition during sterilization and storage. Because of the importance of dextrose infusion fluids in parenteral medicine, it is essential that appropriate methods be established to ensure the quality and shelf-life of these materials. Since 5-HMF is well-known as the principal degradation product of dextrose, a pharmacopeial limit test has been established for "5-HMF and related substances" [13]. This limit test was established with the publication of the Fourth Supplement to USP XIX on 1 May 1978 and is comprised of the direct measurement of the absorbance of a portion of appropriately diluted sample solution at 284 nm [14]. The upper limit for the test is 0.25 absorbance units, which corresponds to a 5-HMF concentration of $\sim$2 ppm. The test is also found in monographs of a number of infusion fluids based on dextrose mixtures (e.g. 5% dextrose, 0.45% NaCl) [15,16]. The principal disadvantage of the compendial method is the potential for interference by absorption of other solution species at 284 nm. Dextrose solutions pre-mixed with drug and formulation components have been shown to be problematic for the assay and have necessitated chromatographic approaches or method modifications in order to accurately quantitate 5-HMF [17,18]. For example, Hryncewicz and co-workers showed that bisulfite, an antioxidant employed in many injectable formulations, was a significant source of assay interference [18]. Several researchers have developed reverse-phased HPLC methods with UV detection to quantitate 5-HMF in dextrose solutions and showed significant deviation from compendial method results, particularly at low 5-HMF levels [6,11,16]. The difference in values obtained between chromatographic and USP approaches was attributed to the non-specificity of the latter.

The pharmacopoeial method for the quantitation of dextrose in solution is based on optical rotation, since dextrose is difficult to quantify by absorption methods due to the absence of a chromophore in its structure [13]. A wide variety of chemical methods for the analysis of dextrose in solution have been proposed, including those based on generic methods for detecting reducing sugars such as oxidation by alkaline copper [5]. A number of additional approaches have been advocated, including use of the Folin-Ciocalteu reagent [8], cyanide-initiated oxygen consumption [6], and iodometric titration [4]. Gas chromatography has been successfully applied for the analysis of derivatized sugar mixtures containing dextrose [19]. Furthermore, Garrett et al. employed a series of steps to sequentially convert dextrose to 5-HMF under alkaline conditions to produce an indirect quantitative spectrophotometric assay for dextrose [5].

As the previous discussion alludes, 5-HMF and dextrose in dextrose-containing infusion fluids are separately quantitated by two independent compendial test procedures. The major challenge in developing a single assay to analyze both dextrose and 5-HMF levels in dextrose solutions is the lack of significant UV–visible absorption by dextrose. Detection using a refractive index (RI) detector for both analytes would be a natural choice if not for the sensitivity demanded for monitoring the typically low levels of 5-HMF. Thus, in the present study, we initially turned to pulsed amperometric detection (PAD) as a sensitive and convenient alternative detection strategy for both dextrose and 5-HMF. Due to the mismatch of concentration of 5-HMF and dextrose, as well as the low column loading capacity for anion exchange columns, simultaneous quantitation of both analytes with a single sample concentration using pulsed amperometry proved difficult. Further sample dilution for dextrose analysis was required due to the high concentrations of dextrose typical to commercial dextrose solutions. To simultaneously quantify both dextrose and 5-HMF in a single sample preparation, anion exchange chromatography was successfully employed with a combination of RI and UV detection for the analysis of dextrose and 5-HMF, respectively. Herein, we report on the performance and application of methods based on anion exchange chromatographic separations coupled with either PAD or combined RI and UV–vis detection for the quantitation of dextrose and 5-HMF in dextrose solutions.

2. Experimental

2.1. Materials

5-HMF (99%) was purchased from Aldrich (Milwaukee, WI), D-(+)-dextrose (99.5%) from Sigma (St. Louis, MO) and 50% sodium hydroxide from Fisher (Pittsburgh, PA). All commercial dextrose infusion solutions examined in the study (5% dextrose, 5% dextrose/0.45% NaCl, 2.5% dextrose/0.45% NaCl) were purchased from Baxter (Chicago, IL).

2.2. Assay procedure

2.2.1. Pulsed amperometric detection (PAD)

High performance anion-exchange chromatographic analysis was carried out using a Dionex GP40 chromatography system outfitted with a Dionex ED 40 amperometric detector with gold working and Ag/AgCl reference electrodes. A 4 × 250 mm Dionex CarboPac PA1 anion exchange column was employed for the separation. The mobile phase employed consisted of 50 mM sodium hydroxide and was prepared by diluting a 50% sodium hydroxide solution with thoroughly degassed ultrahigh purity water (18 MΩ) prepared with a NANO pure Infinity water purification system (Barnstead-Thermolyne, Dubuque, IA). A flow rate of 1 ml/min and sample injection volume of 15 μl was employed in all experiments. Samples for 5-HMF and dextrose analysis were prepared by diluting the sample of interest with ultrahigh purity water to method concentrations of 2 and 15 μg/ml, respectively, for analysis. Specific details around method development and optimization, along with an evaluation of key performance criteria are discussed in Section 3.

2.2.2. Refractive index (RI) and UV–visible detection

Analyses employing RI and UV–vis detection were conducted using a HP1100 HPLC system with a combination of a G1315 Diode Array Detector and G1362 RI Detector. A Phenomenex Rezex™ RFQ fast acid column (100 × 7.8 mm) was employed for the separation of dextrose and 5-HMF at 45 °C. The mobile phase was pure water with a flow rate of 1 ml/min. The injection volume employed was 30 μl. The detection wavelength employed for 5-HMF was 284 nm and dextrose was quantitated by RI detection. Samples for 5-HMF and dextrose analysis were prepared by diluting the sample of interest with ultrahigh purity water to an overall dextrose concentration of 5 mg/ml.

2.3. Thermal decomposition sample preparation

A 5% w/v solution of D-(+)-glucose in USP water was prepared, tightly capped in 50 ml sample vials, and heated at 121 °C in a convection oven to examine 5-HMF formation. The formation of 5-HMF was monitored over various periods of time (0.5, 0.75, 1, 1.5, 2 and 2.5 h). Likewise, a 3.3% w/v solution of D-(+)-glucose in USP water was prepared, tightly capped in 50 ml sample vials, and heated at 130 °C for 2 h for use in method development.

3. Results and discussion

3.1. Method development

Over the past several years, various researchers have reported on the use of liquid chromatography with electrochemical detection to quantitate carbohydrates [20–27]. For example, anion-exchange chromatography with PAD was recently examined for the separation and quantitation of a series of carbohydrates in wine and instant coffee samples [20]. PAD seems particularly well-suited for carbohydrate analysis since carbohydrates can be detected and quantified without need for complex derivatization procedures [21–26]. PAD of carbohydrates is a two-step process involving the adsorption of the analyte species onto a platinum or gold working electrode, followed by electrochemical oxidation for analysis. A major historical drawback noted in the literature for use of PAD is the requirement of strongly alkaline conditions for carbohydrate oxidation [27]. Importantly, anion-exchange columns that are stable under high pH conditions have been recently developed and are now routinely available.

In the present study, a strong anion exchange stationary phase (CarboPac PA1 [28]) was used to separate the weakly acidic dextrose and 5-HMF analytes at high pH (pH 12 NaOH). The pulse sequence of amperometric detection is typically comprised of a sequence of three applied potentials. The first potential is used to oxidize the analyte, the second applied potential is used to oxidize/reduce (or a combination) the electrode surface and remove oxidation products, and the third applied potential is used to reduce (or oxidize) the electrode surface in preparation for repeat of the measurement sequence. The timing of the potential application during the pulse sequence is another important consideration. A delay is typically employed upon stepping the potential to oxidize analyte species in order to allow the charging current to dissipate prior to acquiring data. Following the delay, the current signal is integrated as the analyte species is oxidized. Independent experiments or knowledge about the oxidation potential of the analyte species is used to determine the optimal potential step and integration time for analysis. Typically, the optimal potential for amperometry will be similar for a class of compounds in that the analyte responsivity is controlled not by the inherent redox potential of the compounds but by electrochemical catalysis of analyte species' oxidation at the electrode surface.

In the present study, a series of oxidation potentials were explored in order to optimize the pulsed amperometric detector response for low levels of 5-HMF. Since high oxidation potentials will reduce the lifetime of the gold electrode, the oxidation potential range explored was limited to 0–0.15 V. A sample test solution containing 8 μg/ml of 5-HMF and 16 μg/ml of dextrose was tested for its amperometric response across the potential range. Fig. 1 is a plot of the results, showing the peak area count per μg/ml of 5-HMF and dextrose versus electrode potential. As expected, the detector response for dextrose tends to increase with oxidation potential across the range considered, while the detector response for 5-HMF is largely flat with a very slight decrease at potentials higher than 0.05 V across the range explored.

Therefore, a pulse sequence was selected in the present case (Fig. 2) to achieve suitable signal-to-noise and reproducibility for analysis of low levels of 5-HMF. The gold electrode was equilibrated for 0.2 s at 0.05 V to allow for dissipation of the charging current, followed by signal integration for 0.2 s at 0.05 V. The potential was quickly decreased to −2 V and held for 0.01 s to reductively clean the working electrode, followed by increasing the electrode potential to 0.6 V to oxidize the working electrode surface. This sequence of secondary pulses yielded excellent reproducibility and background currents. The electrode potential was then decreased to −0.1 V to reduce the working electrode surface back to gold. The integration time and sampling potential were optimized to meet the requirements of analysis of both dextrose and 5-HMF in dextrose-containing infusion fluids so that both sample types could be run on a single general method. Due to the extremely low levels of 5-HMF observed and very high concentration of dextrose in many dextrose injections—the concentration

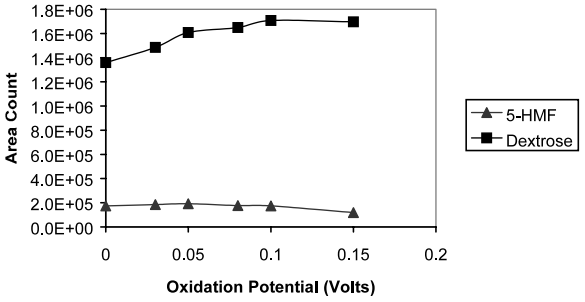

Fig. 1. Pulsed amperometric detector response for 5-HMF and dextrose per μg/ml of each analyte at various oxidation potentials. Lines are provided on the plot to guide the eye.

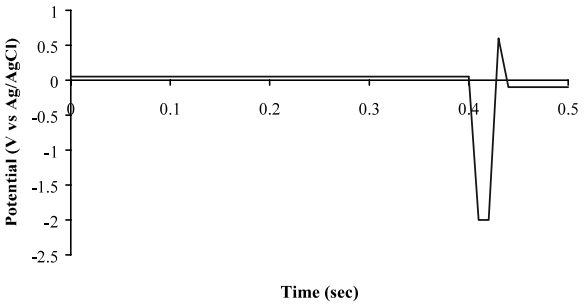

Fig. 2. Optimal pulse sequence employed for amperometric detection.

difference between the two could be as high as 10 [5]—simultaneous quantitation of 5-HMF and dextrose is not practical for many solution sample types. In many cases, column (and detector) overloading for dextrose is observed at the expense of 5-HMF sensitivity. However, use of a common analytical method for both analytes, the conditions for analysis differing only in sample dilution, poses significant advantages over current USP analysis approaches.

As noted, the retention behaviors of analyte species on an anion-exchange column are determined by the properties of the stationary phase, such as the chemical composition of the matrix, functional groupings, morphology of the stationary phase, and ion-exchange capacity. In the present case, the partially ionized dextrose (at pH 12) and 5-HMF analytes bind to quaternary amine groups on the stationary phase and separation is controlled by the specific affinity of the analytes with the quaternary amine functional group. Neutral and cationic compounds will elute in the void volume and would not be expected to interfere with the assay of dextrose and 5-HMF. The degree of retention is expected to be inversely proportional to the analyte pK_a value, and indeed this was observed with dextrose eluting later than 5-HMF. An example analysis chromatogram of a thermally degraded 3.3% dextrose solution is shown in Fig. 3. The retention time of 5-HMF and dextrose was found to increase with a decrease in sodium hydroxide concentration. Note that the full chromatogram in Fig. 3 shows a tiny 5-HMF peak (RT = 3.8 min), followed by dextrose (RT = 5.6 min), and fructose (RT = 6.35 min). The identity of the fructose peak within the chromatogram was confirmed by spiking fructose into a standard solution and by the literature [29,30]. The literature teaches that dextrose degrades to fructose to some extent at elevated temperatures. Consistent with these previous findings, no fructose was observed in unstressed samples while the levels tended to grow with thermal stressing and small quantities of fructose were also observed in commercial dextrose preparations. The inset shown in the upper left of the figure is an expanded view of the 5-HMF peak and shows that adequate sensitivity is obtained, despite the small quantity of 5-HMF present in the sample (87.6 ppm).

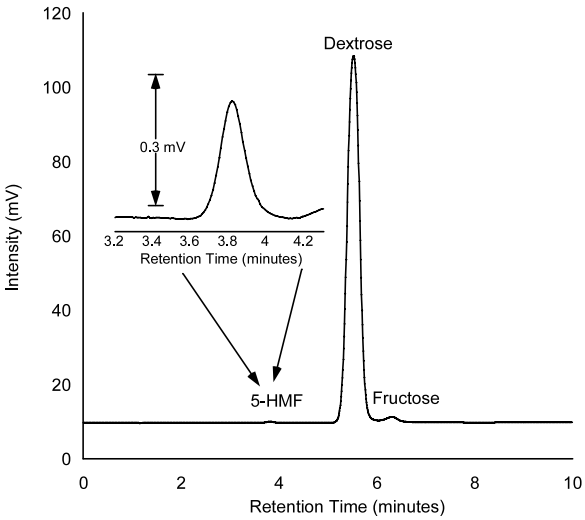

Fig. 3. Typical chromatogram of 3.3% dextrose sample heated at 130 °C for 2 h to produce 5-HMF.

Due to the very low levels of 5-HMF in commercial dextrose solutions, as well as the low sample loading capacity for typical anion exchange columns such as the CarboPac PA1, column and/or detector overloading for dextrose is often observed under conditions of maximal 5-HMF sensitivity. Efforts to optimize the pulsed amperometric detector response to be less sensitive to dextrose proved problematic in that the overall sensitivity of the detection method is higher for dextrose than 5-HMF because of the presence of fewer hydroxyl groups in 5-HMF. Since 5-HMF has an absorbance maxima at 284 nm, a combination of RI for dextrose and UV detection for 5-HMF was explored as an alternative approach. This dual detection scheme approach appeared viable since reduced sensitivity for dextrose at an RI detector was acceptable due the large concentrations typically observed in commercial diluents. For this analysis, we turned to a Phenomenex Rezex™ RFQ Fast Acid column (100 × 7.8 mm) due to its improved capacity over the CarboPAC PA1 and since separation at this column could be achieved with pure water as the mobile phase. As with the PAD-based method, the analysis involves a separation step of 5-HMF and dextrose analytes

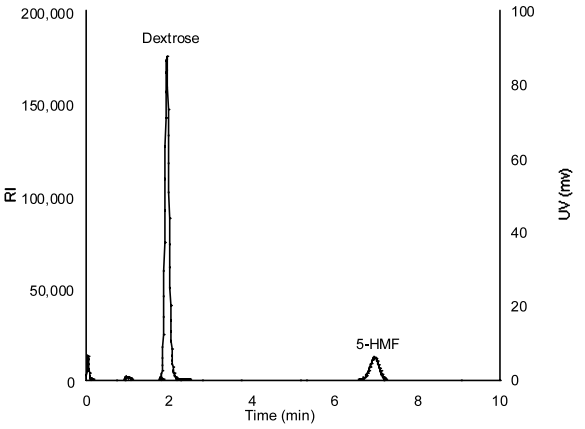

Fig. 4. Representative chromatogram of 5% dextrose injection by RI and UV detection.

at an anion-exchange column. Fig. 4 shows an example chromatogram illustrating the separation and quantitation of dextrose and 5-HMF at 45 °C using two separate detection schemes. As the chromatogram in Fig. 4 shows, both analytes are readily separated and quantitated at a single sample concentration. Due to the additional separation modes beyond purely ion-exchange offered by the Rezex™ column, the order of elution of the two analytes is reversed. Besides offering a single sample preparation for the simultaneous quantitation of both analytes, another potential advantage is that UV and RI detectors are more readily available than PAD-based detection systems.

3.2. Evaluation of method performance

With method parameters established to provide adequate selectivity and optimized detector sensitivity using two separate method approaches, the performance of each approach was examined. Tables 1 and 2 summarize data from a series of experiments designed to assess the linearity, accuracy, and precision of the PAD-based method. Table 1 details dextrose measurement results. The linearity of the amperometric detector response was determined in duplicate from a method concentration ranging from 7.5 to 22.5 μg/ml by analyzing solutions spiked with various amounts of dextrose. Excellent linearity, demonstrated by a slope of 0.99 and a correlation coefficient $r^2 > 0.99$, was achieved for duplicate sample analyses. Mean recovery for dextrose ranged from 98.9 to 101.7%.

Likewise, similar experiments were performed to assess the performance of the same method in relation to 5-HMF and results are reported in Table 2. The linearity of the amperometric detector response was determined in duplicate from a method concentration of ranging from the LOQ (0.05 μg/ml) to 3.0 μg/ml, by analyzing solutions spiked with various amounts of 5-HMF. Excellent linearity, demonstrated by a slope of 1.01 and a correlation coefficient $r^2 > 0.99$, was achieved for duplicate sample analyses. Mean recovery for 5-HMF ranged from 99.0 to 101.0%. The limit of quantitation for 5-HMF was established at 50 ppb (0.05 μg/ml) at a signal-to-noise ratio of 10.

A set of performance criterion were also likewise examined with the dual detection scheme method. The measurement linearity for dextrose analysis by RI was evaluated ranging from typical method concentrations of 10 mg/ml to 10 μg/ml. Excellent linearity was achieved across the measured range with a measurement slope of 0.99 and a correlation coefficient $r^2 > 0.99$. Satisfactory method precision was also obtained with an R.S.D. of 0.1% on ten replicate injections of 5 mg/ml dextrose solutions. Meanwhile, excellent linearity for 5-HMF by UV detection was demonstrated from the LOQ (0.03 μg/ml) to 50 μg/ml with a measurement slope of 0.99 and a correlation coefficient $r^2 > 0.99$. The limit of quantification for 5-HMF was found to be 30 ppb (0.03 μg/ml) at a signal-to-noise ratio of 10.

3.3. Analysis of 5-HMF and dextrose in dextrose samples subjected to thermal degradation by UV and RI detection, respectively

The experiments described in the preceding section indicate that either method approach would be suitable for analysis of 5-HMF and dextrose levels in dextrose solutions. An application of the RI/UV dual detection scheme approach was explored with a representative sample set. To examine the performance of the method in assessing the growth of 5-HMF with thermal exposure, a 5% dextrose solution was prepared in-house and

Table 1
Results of experiments designed to assess method performance of PAD scheme for dextrose analysis

Dextrose spiked, (μg/ml)	Recovery (%)	R.S.D. (%)	Repeatability of 100% method R.S.D. (%) ($n = 10$)	Repeatability of 10% method R.S.D. (%) ($n = 10$)
7.5	101.7	0.6	1.4	4.8
11.25	100.0	0.2		
15	99.3	1.9		
18.75	101.2	2.9		
22.5	98.9	1.1		

incubated at 121 °C for 0.5, 0.75, 1, 1.5, 2 and 2.5 h. The temperature/time profile was chosen to mimic conditions typical for dextrose product sterilization. The degraded dextrose samples were diluted 10-fold and assayed for both 5-HMF and dextrose by UV and RI, respectively. Data from the experiments is reported in Table 3. Clearly, 5-HMF levels observed with the method increase linearly with incubation time, consistent with dextrose degradation under sterilization conditions [8–12]. Note that the extremely low quantities of 5-HMF formed provide little overall change to the dextrose levels observed in the samples in relation to inherent measurement errors. Importantly, the data shows that the method provides adequate sensitivity to quantify the increase in 5-HMF levels with increasing thermal exposure.

3.4. Analysis of 5-HMF and dextrose in commercial dextrose solutions by UV and RI detection, respectively

A series of commercially available dextrose infusion solutions, as well as a freshly prepared dextrose solution were assayed for 5-HMF and dextrose to further examine the utility of the dual detection approach and to compare method performance for 5-HMF to the compendial test method. The results of the experiments are reported in Table 4. The results show that 5-HMF levels in the dextrose solutions varied significantly. 5-HMF level difference amongst the samples is likely due to variation in the sterilization process to which the samples were subjected and storage conditions/durations. As expected, no 5-HMF was detected in freshly prepared dextrose sample. Dextrose levels assayed in all samples were con-

Table 3
5-HMF and dextrose level in 5% dextrose incubated at 121 °C for 0.5, 0.75, 1, 1.5, 2.0 and 2.5 h as determined by UV and RI, respectively

Time Point (h)	5-HMF (ppm)	Dextrose recovery (%)
0.5	< LOQ	98.5
0.75	0.3	99.7
1.0	0.4	97.9
1.5	0.6	98.7
2.0	1.1	98.9
2.5	2.0	97.7

Table 2
Results of experiments designed to assess method performance of PAD scheme for 5-HMF analysis

5-HMF spiked, (μg/ml)	Recovery (%)	R.S.D. (%)	Repeatability of 100% method R.S.D. (%) ($n = 10$)	Repeatability of 10% method R.S.D. (%) ($n = 10$)
1	99.9	5.5	1.3	4.3
1.5	100.5	3.4		
2	99.0	2.3		
2.5	100.0	0.7		
3	101.0	0.3		

Table 4
5-HMF and dextrose levels measured in various commercial dextrose preparations as determined by UV and RI, respectively[a]

Dextrose injection	5-HMF (ppm)	Dextrose (%)	5-HMF and related substance by UV at 284 nm (ppm)[b]
5% Dextrose	7.2	4.8	6.3
5% Dextrose+0.45% NaCl	9.0	4.7	8.3
2.5% Dextrose+0.45% NaCl	3.6	2.4	3.3
Freshly prepared 5% dextrose (non-autoclaved)	N/D	5.0	N/D

[a] Average of two injections.
[b] USP method for 5-HMF and related substances.

sistent with sample label claim. A comparison of 5-HMF values obtained using the subject method and the USP approach was made and results from these experiments are also reported in Table 4. The results show that slightly higher values of 5-HMF are obtained with the subject method compared with the compendial test method, but these differences may simply result from the imprecision of the methods.

4. Conclusions

We have shown that two separate method approaches can be used to separate and quantify 5-HMF and dextrose in commercial dextrose solutions. Common to both approaches is the use of anion-exchange chromatography to separate the analyte species. The approaches differ in detection schemes employed. With PAD, both analytes can be quantitated using pulsed amperometry but different sample preparations are required owing to the concentration mismatch typical in commercial dextrose preparations. This concentration mismatch means that 5-HMF is difficult to quantify at dilutions required for accurate dextrose measurement. Use of a combination of RI for dextrose and UV detection for 5-HMF allows for simultaneous determination of both dextrose and 5-HMF on a single sample preparation. Both methods were shown to exhibit excellent accuracy, linearity, and sensitivity to serve as a replacement for current compendial approaches for 5-HMF and dextrose analysis. These two method approaches provide quick and simple assay of both 5-HMF and dextrose in the various dextrose injections and provides sufficient selectivity for 5-HMF to provide superior specificity relative to the current USP spectrophotometric assay. Application of the dual detection scheme approach was demonstrated by the representative analyses of thermally degraded and commercial dextrose solutions.

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