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Quantitative determination of trace levels of hydrogen peroxide in crospovidone and a pharmaceutical product using high performance liquid chromatography with coulometric detection

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ABSTRACT

A reliable and reproducible high performance liquid chromatography method with coulometric detection was developed and validated for the quantitative determination of trace-levels of hydrogen peroxide in crospovidone, a pharmaceutical excipient, and a capsule pharmaceutical product. The method conditions included: a reproducible extraction procedure to provide a concentrated extract, aqueous extraction solvent; a simple HPLC mobile phase (aqueous 50 mM ammonium acetate) compatible with the coulometric detection; a reserve-phase HPLC column that did not collapse under 100% aqueous mobile phase conditions providing sufficient retention and separation of hydrogen peroxide from interferences; and a coulometric detector with a multi-electrode array providing sensitive and selective detection. The method validation results, including those for specificity, linearity, accuracy, precision, and recovery, were acceptable for the determination of trace levels of hydrogen peroxide. The method was shown to be linear over the range of 0.6–4.5 ppm (μg/g) and 6–90 ppm (μg/g) for the pharmaceutical product and crospovidone, respectively. The described method was applied to the determination of trace levels of hydrogen peroxide in different batches of crospovidone and the corresponding pharmaceutical product batches manufactured from these batches of this excipient.

1. Introduction

Pharmaceutical products are a complex mixture of active pharmaceutical ingredient(s) (API), excipients, and impurities arising from both the API and excipients. Trace impurity levels of hydrogen peroxide (H_2O_2) have been observed to catalyze the instability of pharmaceutical products via a reaction between either the API and excipients, excipient and excipient, or between impurities (Hartauer et al., 2000; Crowley and Martini, 2001; Huang et al., 2003). For example, hydrogen peroxide can oxidize functional groups, such as the double bond of an α,β-unsaturated ketone to an epoxide, an amine to an amine N-oxide or a hydroxylamine and a thiol to a sulfoxide (Waterman et al., 2002; Wasylaschuk et al., 2007). Additionally, hydrogen peroxide can be reduced to the more reactive hydroxyl radical in the presence of catalyzed metal and initiate a radical chain reaction with the API (Sheldon and Kochi, 1976; Johnson and Gu, 1988; Hovorka and Schöneich, 2001; Waterman et al., 2002).

Hartauer et al. (2000) have reported a strong correlation between hydrogen peroxide levels in crospovidone and the extent of degradation of Raloxifene hydrochloride to its N-oxide degradant when exposed to accelerated storage conditions. Crospovidone is a synthetic cross-linked polymer of N-vinyl-2-pyrrolidone and is widely used as a disintegrant in oral solid dosage pharmaceutical formulations (Kibbe, 2000). Hydrogen peroxide is used in the manufacturing process of crospovidone as an initiator for the free-radical polymerization (Witteler and Gotsche, 1999), thus it is highly probable that batches of crospovidone will contain trace levels of hydrogen peroxide as a process impurity. In addition to the potential of trace levels of hydrogen peroxide arising from excipients in a pharmaceutical product, hydrogen peroxide can also be formed as a by-product of free-radical auto-oxidative degradation processes of either the excipients or the API (Hovorka and Schöneich, 2001; Waterman et al., 2002).

Excipient compatibility studies are performed early in the development of a new drug product formulation to assess the instability of the API in the presence of the potential excipients and to characterize the degradation products formed by their interaction to understand how to adequately stabilize the formulation. APIs that are susceptible to oxidative degradation are likely to be sensitive to the presence of trace levels of hydrogen peroxide that may be introduced to the formulation as a trace level impurity in one or more of the excipients used. The purpose of the present study was to establish a specific, sensitive, reproducible and reliable

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analytical method for the quantitation of hydrogen peroxide in a pharmaceutical product under development that was susceptible to oxidative degradation. One of the excipients used in the formulation, crospovidone is known to be a potential source of trace levels of hydrogen peroxide, therefore the present study also included the examination of formulation excipient.

A search of the literature uncovered no reported studies describing the quantification of trace-levels (ppm) of hydrogen peroxide in oral solid dosage pharmaceutical products. However, the literature had noted the utilization of several techniques, including UV/fluorescence detection (Hwang and Dasgupta, 1985; Lazrus et al., 1985, 1986; Masuoka et al., 1996; Zhou et al., 1997; Li and Townshend, 1998; Gay et al., 1999; Schulte-Ladbeck et al., 2003; Pérez and Rubio, 2006) and electrode-based biosensors (Oungpipat et al., 1995; Gundogan-Paul et al., 2002; Anh et al., 2003; Kulys and Tetianec, 2006) for quantifying trace-levels of hydrogen peroxide in the atmosphere (Gunz and Hoffmann, 1990; Lee et al., 2000; Reeves and Penkett, 2003) and biological samples (Tarpey and Fridovich, 2001; Brandes and Janiszewski, 2005; MacDonald-Wicks et al., 2006). The reported UV/fluorescence detection methods typically required numerous sample manipulation steps, including derivatization to produce a chromophore or fluorophore to enable detection; the analysis need to be completed within a limited time frame after sample collection to ensure accurate measurement; and several interferences were observed (Anh et al., 2003; Woo et al., 2003). Electrode-based biosensors have been reported to directly measure hydrogen peroxide in matrices by taking advantage of the hydrogen peroxide redox reactions occurring at the working electrode (Oungpipat et al., 1995; Anh et al., 2003). Although peroxidase/catalase-based biosensors have been shown to provide the selectivity to differentiate peroxides from other electro-active species, peroxidase and catalase have been noted to also react with some organic hydroperoxides (Lazrus et al., 1985, 1986; Magner and Klibanov, 1995; Oungpipat et al., 1995).

Several applications of trace level hydrogen peroxide determination in pharmaceutically relevant excipients have been previously reported in the literature. A ferrous oxidation-xylenol orange with catalase method using UV detection was used for the determination of hydrogen peroxide indirectly in crospovidone (Wasylaschuk et al., 2007). In this method, the amount of hydrogen peroxide in crospovidone is calculated as the difference in total and organic peroxides. The method involves multiple reaction steps, including reacting the sample with a ferrous reagent twice (with and without catalase) to determine organic peroxides and total peroxides. Moreover, this method is not reactive to alkyl peroxides and may generate positive responses for non-peroxides impurities in crospovidone. Additionally, the accuracy of this method is limited by the instability of the test solution, which is stable for only a few hours (Gay et al., 1999). Huang et al. (2003) measured ppm levels hydrogen peroxide in crospovidone using a liquid chromatographic method with amperometric-electrochemical detection. However, the hydrogen peroxide peak eluted rapidly from the LC column, similar to flow injection analysis, as a result the sample required a 100-fold dilution with the mobile phase to minimize quantitation interferences caused by the solvent peak, thus greatly sacrificing the sensitivity of the method. In addition, when their method was applied to pharmaceutical product samples in our laboratory, a significant unidentified peak in the sample matrix was observed to co-elute with the hydrogen peroxide peak. Therefore, the method described by Huang et al. was determined to be unacceptable for the analysis of the pharmaceutical product samples due to the limited selectivity and sensitivity that the separation provided and the inability to generate a true negative control.

Despite the limited selectivity of the method when compared to the other methodologies noted in the literature, the use of liquid chromatography–electrochemical detection does offer some potential advantages including: (1) better specificity via chromatographic separation, (2) simplicity by direct measurement without derivatization, and (3) adequate sensitivity. One of the objectives of this work was to develop a sensitive and direct method for the determination of trace levels of hydrogen peroxide in a pharmaceutical product under development, which contains an API with an amino group in the molecule which is a potential oxidation target, and several different common excipients, including crospovidone, lactose monohydrate, and magnesium stearate. HPLC coupled with amperometric-electrochemical detection has been reported to detect hydrogen peroxide using platinum electrodes (Huang et al., 2003; Sittampalam and Wilson, 1983) via oxidation ($H_2O_2 \rightarrow 2H^+ + O_2 + 2e^-$). However, since the amperometric detector operates by detecting electrochemical reactions that take place in thin-layer cells, it has several disadvantages (Cunico et al., 1998), including (1) only a fraction (less than 10%) of the molecules contact the electrode and undergo the redox reaction; (2) the signal is flow sensitive; and (3) frequent cleaning and long electrode stabilization time is required prior to analysis (Huang et al., 2003). With coulometric detection, the eluent flows through a porous graphite carbon electrode (which possesses an enlarged surface area) in contrast to amperometric detection where the eluent flows past the electrode (Scott, 1996); as a result, the coulometric signal is independent of flow rate and remains unaffected by surface poisoning until more than 90% of the electrode has fouled (ESA Inc., 2006). The latter feature eliminates the need for electrode maintenance during extended periods of use (Ouchi and Watanabe, 2002) making coulometric detection more suitable for routine analysis. In addition, analyte sensitivity can be enhanced using a detector equipped with an electrode array connected in series (Scott, 1996). Therefore, to determine trace levels of hydrogen peroxide in excipients and pharmaceutical products, a liquid chromatography method combined with coulometric electrochemical detection (LC–CECD) was investigated.

A specific, sensitive, and reproducible LC–CECD method for the quantitation of hydrogen peroxide in crospovidone and a pharmaceutical drug product containing crospovidone as the major excipient is reported in this paper. A reproducible extraction procedure was developed to provide a concentrated extract; electrochemical detection compatible HPLC conditions were developed to provide sufficient resolution of hydrogen peroxide from potential interferences; and coulometric-electrochemical detection was applied to provide the desired sensitivity and a multi-graphite electrode array configuration was utilized to provide additional selectivity. Validation, including specificity, linearity, accuracy, precision, and recovery, was performed using matrices of crospovidone and an encapsulated drug product. The described procedures offers significant advantages over previously published methodologies for detecting trace level (ppm) of hydrogen peroxide.

2. Methods

2.1. Chemicals and materials

Hydrogen peroxide solution (≥30% for trace analysis) was purchased from Fluka (Steinheim, Switzerland). Ammonium acetate (purity ≥ 99.99%) was obtained from Aldrich (Milwaukee, WI, USA). Water was deionized and further purified using a Nanopure Diamond® water purification system from Barnstead International (Dubuque, IA, USA). Disposable glass tubes (16 mm × 100 mm) of Durex™ borosilicate, Nylon Acrodisc® Syringe Filters (13 mm, 0.2 μm), syringe (3 mL, 0.8 mm × 25 mm, Becton Dickinson & Co.) and disposable scintillation vial (20 mL) of borosilicate glass were purchased from VWR (West Chester, PA, USA). Crospovidone (polyplasdone XL-10) was supplied from International Specialty Products (Wayne, NJ, USA). Encapsulated product samples were

supplied by Bristol-Myers Squibb containing active pharmaceutical ingredient (63%), and inactive ingredient crospovidone (6%), lactose monohydrate (30.6%), and magnesium stearate (0.4%).

2.2. Liquid chromatography–coulometric electrochemical detection

A Waters Alliance Model 2695 HPLC system (Milford, MA, USA) equipped with a quaternary pump, an autosampler, a column thermostat and an ESA Model 5600A CoulArray® Detector (Chelmsford, MA, USA) was used for LC separation and detection. An isocratic chromatographic separation was performed on an Atlantis® dC18 column (4.6 mm × 150 mm, 3 μm particle size) (Milford, MA, USA) at ambient temperature. The mobile phase consisting of 50 mM ammonium acetate in water was delivered at 1 mL/min. The injection volume was 5 μL. The column effluent was pumped to a coulometric detector equipped with palladium reference electrodes and an array of eight porous graphite working electrodes. The detector was operated in oxidation mode. The eight electrode potentials were set at 0 mV, 125 mV, 250 mV, 375 mV, 500 mV, 625 mV, 750 mV, and 875 mV for four minutes followed by 1 min clean cell cycle. Each run was 5 min long. Data acquisition was accomplished using CoulArray Ver 1.12 (ESA, Inc.). Quantification of hydrogen peroxide was performed using the data generated at 500 mV potential.

2.3. Standard solution preparation

A stock solution of hydrogen peroxide (1000 μg/mL) was prepared in water and calibration curves were constructed to bracket the expected concentrations of hydrogen peroxide in samples. The calibration standard solutions for crospovidone ranged from 1 μg/mL to 15 μg/mL. A series of corresponding working standard solutions containing 1 μg/mL, 2 μg/mL, 4 μg/mL, 6 μg/mL, 8 μg/mL, 10 μg/mL, 12 μg/mL, and 15 μg/mL hydrogen peroxide were prepared by diluting the stock standard solution with water.

Calibration standard solutions for the pharmaceutical product samples ranged from 0.2 μg/mL to 1.5 μg/mL. A series of corresponding working standard solutions containing 0.2 μg/mL, 0.4 μg/mL, 0.6 μg/mL, 0.8 μg/mL, 1.0 μg/mL, 1.2 μg/mL, and 1.5 μg/mL hydrogen peroxide were prepared by diluting the stock standard solution with water.

2.4. Sample preparation

2.4.1. Excipient

Crospovidone sample (~1 g) was accurately weighed and transferred to a 20-mL glass vial, and 6.0 mL of water was added. The mixture was vortexed until homogenous, then sonicated for 5 min. The mixture was then briefly vortexed again, poured into a tube, and centrifuged at 3000 rpm for 20 min. The supernatant was filtered with a 0.2 μm nylon syringe filter to obtain a clear solution.

Samples for recovery were prepared by substituting the water with hydrogen peroxide working standard solutions containing 1 μg/mL, 6 μg/mL, or 12 μg/mL of hydrogen peroxide.

2.4.2. Pharmaceutical product

The contents from several capsules were pooled and a sample (~1 g) was then accurately weighed, transferred to a 20-mL vial, and 3.0 mL of water was added. The mixture was vortexed until homogenous, then sonicated for 5 min. The mixture was then briefly vortexed again, poured into a tube, and centrifuged at 3000 rpm for 20 min. The supernatant was filtered with a 0.2 (m nylon syringe filter to obtain a clear solution.

Samples for recovery were prepared by substituting the water with hydrogen peroxide working standard solutions containing 0.4 μg/mL, 0.8 μg/mL, or 1.2 μg/mL of hydrogen peroxide.

2.5. Recovery

Recovery of the method was determined at three concentration levels of working standard solution, 1 μg/mL, 6 μg/mL, and 12 μg/mL for the crospovidone samples, and 0.4 μg/mL, 0.8 μg/mL, and 1.2 μg/mL for the pharmaceutical product samples.

At each concentration investigated, triplicate sample preparations were prepared using three unique hydrogen peroxide working standard solutions. In addition, unspiked sample solutions were prepared in triplicate for each sample matrix. The recovery was evaluated by comparing the average response of the spiked sample solution (after correcting for the average response of the unspiked sample solution) to the average response of the working standard solution.

2.6. Calculation

The amount of hydrogen peroxide present in the crospovidone and pharmaceutical product samples was calculated using the corresponding calibration curve and corrected for extraction volume, sample weight and average sample matrix recovery, determined in Section 2.5.

3. Results

While developing a suitable method to evaluate the hydrogen peroxide levels in crospovidone and pharmaceutical products, challenges were encountered to achieve sufficient limit of quantification (LOQ) and selectivity from potential interferences to reproducibly quantitate ppm (w/w) levels of hydrogen peroxide. The initial separation conditions did not adequately retain hydrogen peroxide on the column creating interference and insufficient resolution from the peaks of sample matrix and system void volume. Several steps were taken to resolve these issues. First, an extraction procedure was developed to provide sufficient accuracy, reproducibility and limit of quantification by generating concentrated extracts of the crospovidone and pharmaceutical product sample matrices. Second, the HPLC and coulometric-electrochemical detection conditions were modified to better resolve the hydrogen peroxide peak from those of the sample matrix and the system void volume. Third, the LOQ provided by the coulometric detector for hydrogen peroxide was evaluated. A LOQ at 0.2 ppm of hydrogen peroxide was achieved. After resolving these issues, method validation (including specificity, linearity, recovery, precision, and accuracy) was performed for hydrogen peroxide in both the crospovidone and capsule pharmaceutical product matrices in accordance with current guidances for pharmaceutical analysis (Ermer and Miller, 2005). The drug product formulation investigated contained 6% crospovidone and 63% active pharmaceutical ingredient (API).

3.1. Sample extraction

Water was chosen as the extraction solvent, as hydrogen peroxide is freely soluble in water, however, basic conditions were avoided as hydrogen peroxide is generally less stable under these conditions (Zhang and Wilson, 1993; Merck & Co. Inc., 2006; US Peroxide, 2006]. The volume of water used during the extraction was optimized to fully wet the sample while also ensuring a concentrated extract could be obtained. Since the density of commercial pharmaceutical grade crospovidone is typically around 0.5 g/cm^3 (Kibbe, 2000), significantly lower than the density of water (1.0 g/cm^3), a 1 g sample was weighed into the container before

adding water for extraction. This prevented the crospovidone from floating on top of the water. Experimentation demonstrated that if the components were not added in this order the mixture could not be adequately homogenized (complete wetting of the entire crospovidone sample) even after extended vortexing times. For the crospovidone samples, it was determined that 6.0 mL of water was necessary to wet the entire sample due to its insolubility in water and lower bulk density. The presence of other excipients and the API in the formulation enhanced the wetability of the pharmaceutical product investigated. As a result, it was determined that only 3.0 mL of water was required to wet the entire sample. To maximize the concentration of hydrogen peroxide in solution a minimum extraction solvent volume was used, which resulted in a thick slurry requiring a centrifugation step to obtain a clear supernatant for analysis. A syringe equipped with a water compatible filter (0.2-μm hydrophilic nylon membrane) was used to obtain a particle free sample of the supernatant for LC–CECD analysis.

3.2. Liquid chromatography–coulometric electrochemical detection

The chromatographic conditions described by Huang et al. (2003) were evaluated. However, it was noted that under these conditions the hydrogen peroxide peak eluted with the solvent front, similar to flow injection analysis as described by the authors. Quantitation of hydrogen peroxide was challenging even in standard solutions as the peak of interest co-eluted with the system and void volume peaks. Furthermore, a large unidentified peak associated with the pharmaceutical product was observed to co-elute with the hydrogen peroxide peak under these conditions when the pharmaceutical product sample was evaluated. To improve the retention of hydrogen peroxide, the mobile and stationary phases were modified. The mobile phase was switched from pH 8 buffer to a neutral buffer, which is highly compatible with the stability of the hydrogen peroxide. A reversed-phase column, with a stationary phase did not collapse under 100% aqueous mobile phase conditions and provided longer retention for polar compounds, was investigated to improve the retention of hydrogen peroxide. Conditions were optimized using an Atlantis™ dC18 column (3-μm, 4.6 mm × 150 mm) and an aqueous mobile phase containing 50 mM ammonium acetate. These modifications resulted in better separation of hydrogen peroxide from the interference peaks observed in both the excipient and pharmaceutical product sample solutions than was achieved with the previously reported conditions, as illustrated in Fig. 1.

Additional selectivity was achieved, particularly for pharmaceutical product sample solutions, using a coulometric detector with a multi-working electrode array in series. Potentials from 0 mV to 875 mV in equally spaced increments were applied sequentially to each of the eight working electrodes in oxidation mode vs. a palladium reference electrode. As shown in Fig. 2, the chromatogram in the 500 mV potential channel offered the most specific detection, compared to the other potentials investigated, minimizing interferences from the other components in the pharmaceutical sample matrix. Thus, only the data generated at 500 mV potential was used for quantification although other seven potentials applied before and after 500 mV were required to achieve the specificity of the method.

Representative LC–CECD chromatograms of hydrogen peroxide in standard, crospovidone and pharmaceutical product sample solutions are shown in Fig. 3.

3.3. Method validation

3.3.1. Specificity

The specificity of the method was evaluated by comparing blank, standard, and spiked excipient/pharmaceutical product sample solutions. As shown in Fig. 4, the absence of hydrogen peroxide peak in the blank, and a corresponding increase in the hydrogen peroxide signal in the sample solutions spiked with increasing con-

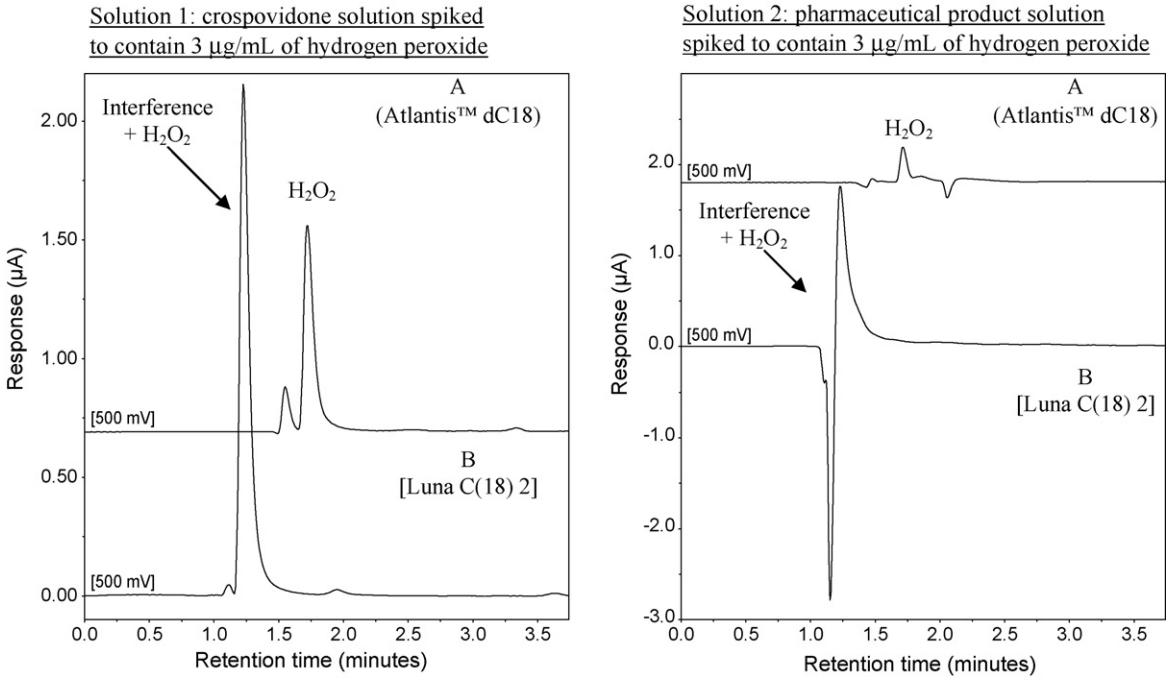

Fig. 1. Improved separation of hydrogen peroxide from an interference peak was obtained by changing the stationary and mobile phases. Experimental conditions: per procedure described in Section 2.4.1. (Excipient) and Section 2.4.2. (Pharmaceutical product), Solution 1 was prepared by extraction of 1 g crospovidone sample with 6.0 mL of 3 μg/mL of H_2O_2 solution, and Solution 2 was prepared by extraction of 1 g capsule content with 3.0 mL of 3 μg/mL of H_2O_2 solution; (A): column: Atlantis™ dC18, 150 mm × 4.6 mm, 3 μm; mobile phase: 50 mM ammonium acetate; flow rate: 1 mL/min; column temperature: ambient; (B) conditions used in Ref. [3]: column: Luna C(18)2, 150 mm × 4.6 mm, 5 μm; mobile phase: 50 mM KH_2PO_4, pH 8; flow rate: 1 mL/min; column temperature: ambient.

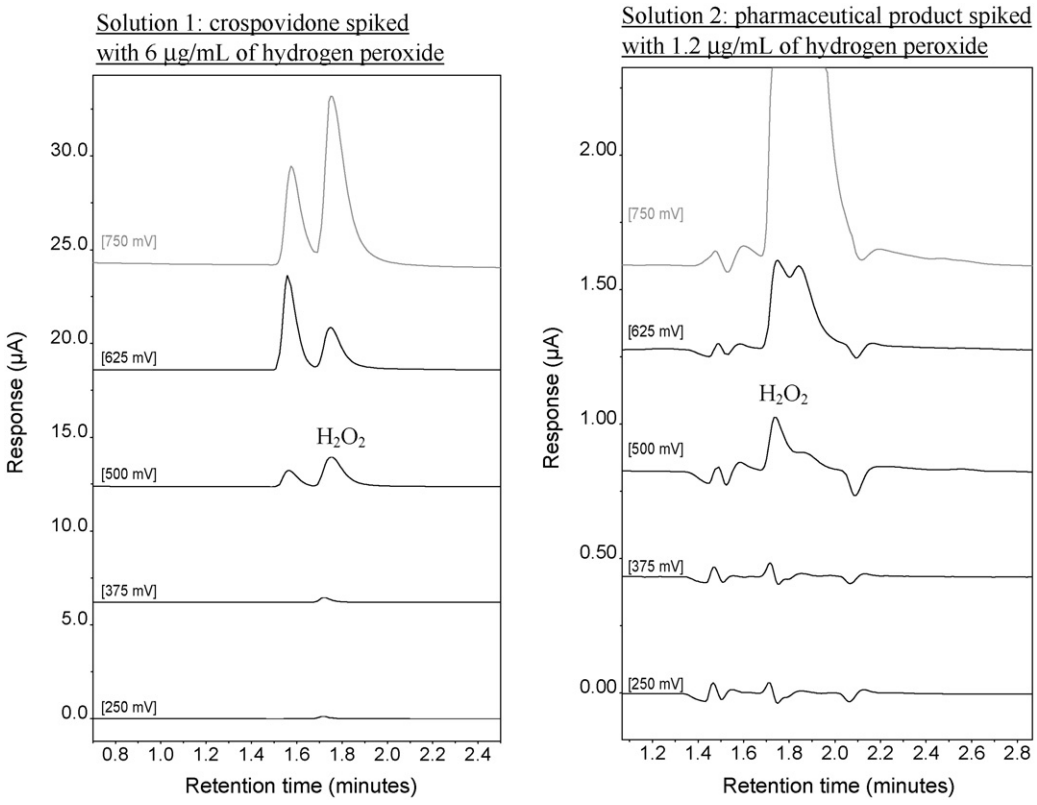

Fig. 2. LC–CECD chromatograms of spiked crospovidone and pharmaceutical product samples. The overlays were generated by monitoring eight different potential channels of the coulometric array detector. The 500 mV channel was selected for quantitation as it provided excellent sensitivity and selectivity. Experimental conditions: per procedure described in Section 2.4.1. (Excipient) and Section 2.4.2. (Pharmaceutical product), Solution 1 was prepared by extraction of 1 g crospovidone sample with 6.0 mL of 6 μg/mL of H_2O_2 solution, and Solution 2 was prepared by extraction of 1 g capsule content with 3.0 mL of 1.2 μg/mL of H_2O_2 solution.

centrations of hydrogen peroxide demonstrated the specificity of the method.

3.3.2. Limit of quantification (LOQ) and linearity

Five independent standard solutions were prepared containing 0.2 μg/mL of hydrogen peroxide and analyzed using the method described. The LOQ was determined to be 0.2 μg/mL with a 5% R.S.D. (%) of peak response due to hydrogen peroxide.

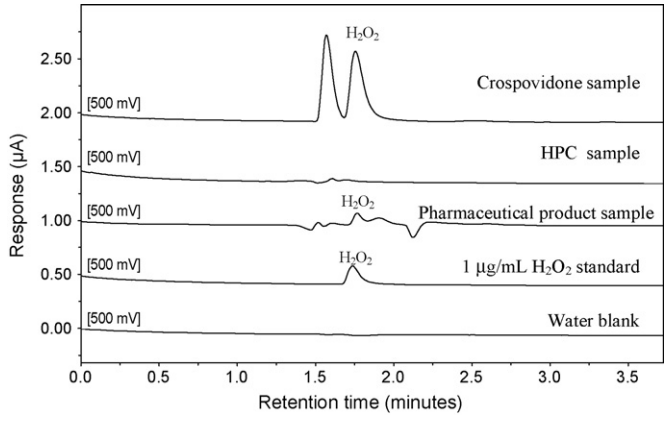

Fig. 3. Overlay of representative LC–CECD chromatograms of (from top to bottom) an unspiked crospovidone sample, an unspiked HPC sample, an unspiked pharmaceutical product sample, an 1 μg/mL hydrogen peroxide standard solution and a water blank. Experimental conditions: per procedure described in Section 2.4.1. (Excipient) and Section 2.4.2. (Pharmaceutical product), crospovidone sample solution was prepared by extraction of 1 g crospovidone sample with 6.0 mL of water; HPC sample was prepared by extraction of 1 g of HPC sample with 18 mL of water; and pharmaceutical product sample solution was prepared by extraction of 1 g capsule content with 3.0 mL of water.

Due to the differences in the range of hydrogen peroxide concentrations expected in the two different sample matrices, unique standard calibration curves were prepared for the quantitation of hydrogen peroxide in crospovidone (1–15 μg/mL) and pharmaceutical product (0.2–1.5 μg/mL). Statistical analysis of the higher concentration crospovidone calibration curve (1–15 μg/mL) demonstrated excellent linearity with a coefficient of determination (R^2) of 0.9998. The statistical analysis of the lower concentration pharmaceutical product calibration curve (0.2–1.5 μg/mL) demonstrated good linearity as well with a coefficient of determination (R^2) of 0.9982.

For comparison, matrix-based calibration curves within the same concentration ranges were constructed by spiking hydrogen peroxide into the crospovidone and the pharmaceutical product samples. The response of hydrogen peroxide for each spiked sample solution was corrected for the response of the unspiked crospovidone or pharmaceutical product sample matrix. These matrix-based calibration curves were prepared following the sample preparation procedure substituting standard solution for the extraction solvent to reflect any biases that may occur during sample preparation. Coefficient of determination values (R^2) 0.9993 and 0.9904 were obtained for crospovidone and pharmaceutical product matrices, respectively, demonstrating an excellent correlation between concentration and response within the sample matrices investigated.

3.3.3. Recovery and reproducibility

The recovery of the method was evaluated by comparing the response of the spiked sample, after correcting for the response of

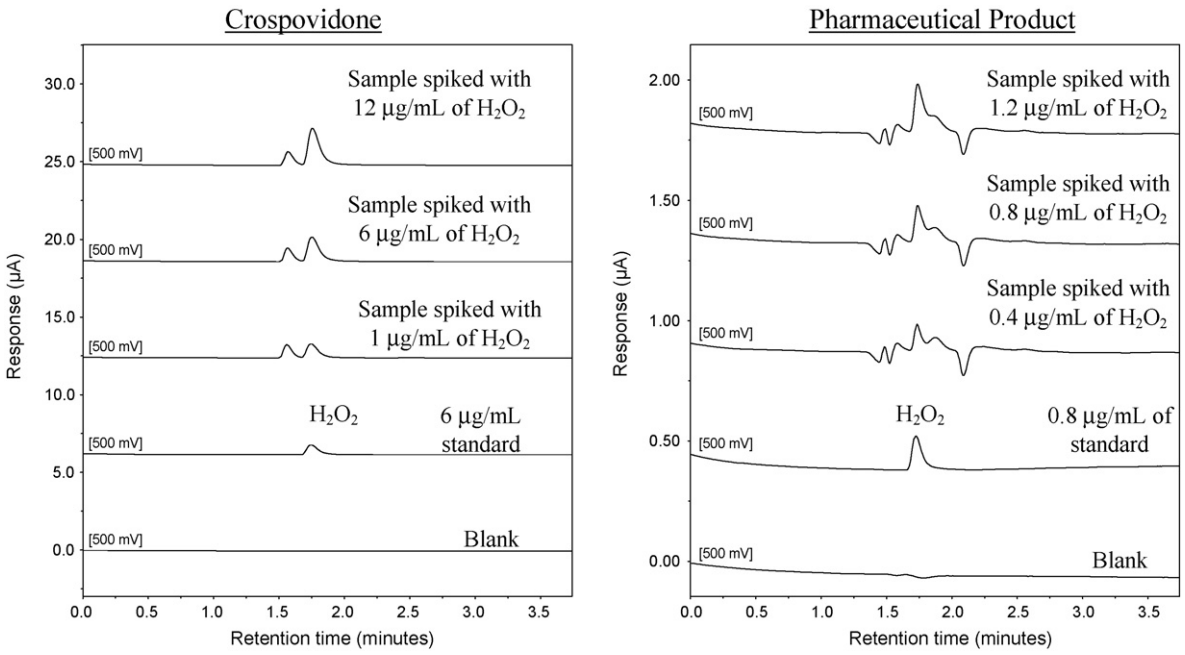

Fig. 4. The specificity of the method is shown in the chromatogram overlays of crospovidone and pharmaceutical product spiked sample solutions. The hydrogen peroxide peak can be easily monitored in both sample matrices. Experimental conditions: per procedure described in Section 2.4.1. (Excipient) and Section 2.4.2. (Pharmaceutical product), crospovidone sample solutions spiked with 12 µg/mL of H_2O_2, 6 µg/mL of H_2O_2, and 1 µg/mL of H_2O_2 were prepared by extraction of each of 1 g crospovidone sample with each of 6.0 mL of 12 µg/mL, 6 µg/mL, and 1 µg/mL of H_2O_2 solutions separately; and pharmaceutical product sample solutions spiked with 1.2 µg/mL of H_2O_2, 0.8 µg/mL of H_2O_2, and 0.4 µg/mL of H_2O_2 were prepared by extraction of each of 1 g capsule content sample with each of 3.0 mL of 1.2 µg/mL, 0.8 µg/mL, and 0.4 µg/mL of H_2O_2 solutions separately.

the unspiked sample, to the response of the standard solution at three concentrations. Each spiked sample solution was prepared in triplicate. The results obtained for both the crospovidone and pharmaceutical product matrices are given in Table 1. Good R.S.D. values (0.4–12%, $n = 3$) were obtained for all concentrations investigated, indicating the extraction procedure was sufficiently reproducible. The recovery of hydrogen peroxide from the pharmaceutical product matrix was reproducible however, it was consistently less than 50%. This is mainly due to using a shoulder-to-shoulder parameter to integrate the hydrogen peroxide peak from a partially co-eluted unidentified shoulder peak. Therefore, a correction factor must be applied when quantifying the amount of hydrogen peroxide in this sample matrix, or sample solutions must be quantified against a matrix-based calibration curve.

3.3.4. Accuracy and precision using matrix-based calibration curve

The accuracy and precision of the method were evaluated by spiking the same batch of crospovidone and pharmaceutical product with hydrogen peroxide in triplicate at three concentrations and determining the spiked concentration against matrix-based calibration curves. The percentage of target concentration (%) served as the measure of accuracy, which was calculated by comparing the found concentration to the expected concentration. The R.S.D. (%) of the triplicate preparations served as the measure of precision. The results are provided in Table 2. Acceptable accuracy (93–104%) and precision (≤4%, $n = 3$) were observed for the trace analysis throughout the linear range for both the crospovidone and pharmaceutical product sample matrices. These results validate the use of matrix-based calibration curves to quantify hydrogen peroxide in crospovidone and the pharmaceutical product of interest.

3.4. Application of the method

The described method was applied to the determination of trace-level hydrogen peroxide in six batches of crospovidone and the corresponding batches of pharmaceutical product. The results are provided in Table 3. For each sample, three independent sample preparations were made. The sample results were calculated using the matrix-based calibration curve. The concentration lev-

Table 1
Recovery of hydrogen peroxide in crospovidone and pharmaceutical product and reproducibility (represented by R.S.D.).

	Spiked concentration (µg/mL)	Recovery[a] (%)	R.S.D. (%)
Crospovidone			
H_2O_2	1	114	6
	6	96	2
	12	89	0.4
Average recovery		100	
Pharmaceutical product			
H_2O_2	0.4	43	12
	0.8	40	7
	1.2	45	3
Average recovery		43	

[a] Mean of three determinations.

Table 2
Accuracy (represented by percentage of target concentration) and precision (represented by R.S.D.) of hydrogen peroxide in crospovidone and pharmaceutical product using matrix-based calibration curve.

	Nominal concentration (µg/mL)	Percentage of target concentration[a] (%)	R.S.D. (%)
Crospovidone			
H_2O_2	1	97	4
	6	100	3
	12	100	0.4
Pharmaceutical product			
H_2O_2	0.4	93	3
	0.8	93	4
	1.2	104	1

[a] Mean of three determinations.

Table 3
Concentrations of hydrogen peroxide in crospovidone and pharmaceutical product calculated using matrix-based calibration curves.

Batch #	Crospovidone		Pharmaceutical product	
	H_2O_2 (μg/g)[a]	R.S.D. (%)	H_2O_2 (μg/g)[a]	R.S.D. (%)
1	22	0.3	1.1	4
2	48	0.3	2.1	6
3	20	2	1.3	5
4	21	3	1.2	9
5	43	1	1.7	5
6	45	1	1.7	0.5

[a] Mean of three determinations.

els of hydrogen peroxide observed in the crospovidone batches ranged from 22 μg/g to 45 μg/g with R.S.D. values ranging from 0.3% to 3%. The concentration levels of hydrogen peroxide observed in pharmaceutical product batches manufactured from these batches of crospovidone ranged from 1 μg/g to 2 μg/g with R.S.D. values ranging from 0.5% to 9% ($n = 3$). The values determined in the product batches were in good agreement with the expected levels of hydrogen peroxide in batch based on the 6% input amount of the crosprovidone batches, indicating a consistent level of carry over despite the numerous processing steps required to complete the manufacturing of the pharmaceutical product. A coefficient of determination (R^2) of 0.8747 was observed between the levels of hydrogen peroxide in the input crospovidone batches and the levels of hydrogen peroxide in corresponding batches of drug product. Thus, for drug products containing APIs that are known to be susceptible to oxidative degradation, the described method is well suited for screening excipients for the presence of trace levels of hydrogen peroxide to ensure the stability of the formulation.

4. Discussion and conclusion

Crospovidone is widely used as a super disintegrant in oral solid dosage pharmaceutical formulations. Trace-levels of hydrogen peroxide introduced from crospovidone can cause degradation of API susceptible to oxidative degradation. Thus, the hydrogen peroxide content of crospovidone can be a critical quality attribute when evaluating the stability of formulations containing this super disintegrant. In the present study, a reliable and reproducible liquid chromatography–coulometric electrochemical detection (LC–CECD) method was developed and validated for the quantitative determination of low ppm (μg/g) level of hydrogen peroxide in crospovidone and a pharmaceutical product. Six pharmaceutical grade batches of crospovidone (polyplasdone XL-10) were tested using this method. The concentration of hydrogen peroxide ranged from 22 to 45 ppm (μg/g). A direct comparison to the previously reported methods referenced in this paper could not be made since the same batches and/or instrumentation was not available. However, since the same crospovidone vendor was evaluated by all laboratories a comparison of the range of results was made. The range of results obtained using the described methodology were consistent with those obtained using the indirect UV detection method described by Wasylaschuk et al., (40–48 ppm) and the liquid chromatographic with amperometric-electrochemical detection method described by Huang et al. (58 ppm).

In comparison to the indirect UV method, the described LC–CECD method directly quantitates hydrogen peroxide eliminating the need for multiple reaction steps to generate the UV chromophore and separate analyses for total and organic peroxide. The LC–CECD method eliminates the interference due to non-peroxide impurities by using chromatography separation; and provides a sample solution that is stable eliminating the requirement to complete the testing rapidly due to the instability of the test solution. In comparison to the liquid chromatography–amperometric electrochemical detection method, the described LC–CECD method provides better separation from interferences arising from the formulation matrix by utilizing improved chromatographic conditions and a commercially available coulometric detector with a multi-graphite electrode array configuration. The LC–CECD method is better suited for routine analysis since the porous graphite carbon electrode possesses an enlarged surface area which is less susceptible and affected to surface poisoning after extended periods of use. Moreover, the reproducible extraction procedure of the LC–CECD method provides a concentrated extract enabling a lower limit of quantitation.

The described procedure offers significant advantages over previously published methodologies, and can be easily adapted for the analysis of other pharmaceutical excipients and oral solid dosage formulations through minor modifications to the extraction procedure. For example, hydrogen peroxide is used during the manufacturing process to reduce the average molecule weight of hydroxypropyl cellulose (HPC) (Kroschwitz et al., 1993), another commonly used pharmaceutical excipient. Therefore trace amount of hydrogen peroxide may remain in the end product. Since HPC is a water soluble polymer that forms a colloidal solution in water at ambient temperature, modification of the volume of water needed (18 mL vs. 6 mL) for extraction and filtration was made. A representative LC–CECD chromatogram of hydrogen peroxide in a HPC sample is shown in Fig. 3. In this particular sample, no hydrogen peroxide was detected. 4–12 ppm of hydrogen peroxide has been previously reported in pharmaceutical grade HPC batches (Wasylaschuk et al., 2007).

The methodology described in this paper is the first published liquid chromatography–coulometric electrochemical detection method for hydrogen peroxide, and is an effective tool for monitoring hydrogen peroxide in excipients and drug product formulations, to ensure their safety and efficacy.

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