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A liquid chromatographic assay for urea in over-the-counter carbamide peroxide products

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Abstract: A liquid chromatographic assay has been developed and validated for determining the amount of urea present in carbamide peroxide formulations. The method employs an interaction CHO-620 carbohydrate column loaded in the Ca^{+2} form, a flow rate of 0.6 ml min^{-1}, a column temperature of 85°C, and UV detection at 200 nm. The mobile and stationary phase variables that may have an effect on the urea separation were studied and are discussed. The method was found to be linear over a range of 20 μg/g to 340 μg/g of urea, with a limit of detection of 1 μg/g and a quantitation limit of 15 μg/g. System precision was found to have a %RSD of less than 1.0% while the method precision was typically less than 5.0%. Recoveries of 97.4% (1.0% RSD) and 98.3% (1.1% RSD) of the theoretical amount of urea (6.6% on a weight to weight basis) contained in two different formulations were obtained.

Keywords: *Urea; carbamide peroxide; reversed-phase chromatography; ion chromatography.*

Introduction

Several analytical methodologies have been developed for determining the amount of urea present in different matrices. A flow injection colorimetric assay was developed for biological samples [1]. The method, which reacts urea with *o*-phthaladehyde and *N*-(1-naphthyl) ethylenediamine, can be run at 37°C or lower and produces a coloured product that absorbs at 505 nm. The reaction, however, is time consuming and the system was not linear.

Liquid chromatographic separations have been developed for the quantification of urea that is present in soil, food and biological samples. These separations have been accomplished using reversed-phase [2, 3], cation exchange [4], anion exchange [5], ligand exchange [6] or ion-pair chromatography [7, 8].

Generally the chromatographic methods have used post-column detection. The post-column system that is typically used is composed of immobilized urease that enzymatically converts urea to ammonia. The resulting ammonia is then reacted with orthophthalaldehyde to form a highly fluorescent or UV absorbing compound [3, 7, 8]. The post-column reactor system, however, is not commercially available and must be prepared by the user. The system also requires that two reactions take place after the elution of urea; first the conversion of urea to ammonia followed by the reaction of ammononia with orthophthalaldehyde to form the fluorescent or UV-active compound. Other detection schemes have also been used including UV detection or refractive index detection [2, 4–6].

The quantification of urea in pharmaceutical formulations is important in order to determine the products' potency and stability. The current analytical methodology for determining the amount of urea in carbamide peroxide formulations is very tedious, is not specific for urea, and is time consuming. One method requires the sample to be heated and the release of ammonia is determined by placing a piece of litmus paper above the test tube and watching for a colour change from red to blue. Another method reacts urea with nitric acid to form a precipitate (urea nitrate). A third method requires the digestion of the sample followed by a back titration with sodium hydroxide.

This paper discusses the separation and direct UV detection of urea in carbamide peroxide formulations. The separation scheme uses a commercially available carbohydrate column (cationic polymer column loaded in the calcium form) and UV detection at 200 nm. The formulations are diluted with water and then injected into the LC system. The urea

assay was found to be linear over a range of 20 μg/g to 340 μg/g with a detection limit of 1 μg/g and a quantitation limit of 15 μg/g. The system precision (%RSD) was less than 1.0% while the method precision (%RSD) was less than 5.0%.

Experimental

Chemicals

HPLC-grade water was obtained from Burdick and Jackson (Muskegon, MI, USA). Ethylenediaminetetraacetic acid calcium disodium salt was obtained from The Aldrich Chemical Company (Milwaukee, WI, USA). Urea was obtained from Mallinckrodt Specialty Chemical Company (Paris, KY, USA). All chemicals were of reagent grade. The carbamide peroxide samples were purchased at local pharmacies.

Apparatus

The instrumentation used in this study consisted of a Spectra–Physics Isochrom pump, Spectra–Physics 8875 autosampler, Spectra–Physics UV100 UV detector, a Spectra–Physics Chromjet Integrator (Thermo Separation, San Jose, CA, USA), an Eppendorf column heater and TC-50 controller (Eppendorf North America, Inc., Madison, WI, USA). The Interaction CHO-620 Carbohydrate column, 6.5 × 300 mm (Interaction Chemicals Inc., Mountain View, CA, USA) was used for the separation. The column is composed of a cation exchange polymer in the Ca^{+2} ionic form. Aqueous analyte samples of about 200 μg/g and sample aliquots of 20 μl were used. Inlet pressures of 800–1100 psi were observed. A column temperature of 85°C was used and a wavelength of 200 nm was used for UV detection.

Mobile-phase preparation

The mobile phase was prepared by weighing out approximately 50 mg l^{-1} of calcium disodium EDTA, transferring it to a 1-l volumetric flask, diluting to volume with HPLC-grade water and mixing. The solution was vacuum filtered through a 0.45-μm PTFE membrane and purged with helium for approximately 10 min.

Standard prepration

Standards were prepared by weighing out approximately 100 mg of urea into a 100-ml volumetric flask, diluting to volume with HPLC-grade water and mixing. The working standards were prepared by transferring an aliquot of the 1000 μg/g standard to an appropriate volumetric flask, diluting to volume with HPLC-grade water and mixing.

Sample preparation

Approximately 100 mg of carbamide peroxide sample was weighed into a 50-ml volumetric flask, diluted to volume with HPLC-grade water and mixed. A portion of the sample solution was transferred to an autosampler vial and injected into the LC system.

Results and Discussion

A liquid chromatographic separation was developed and was found to be suitable for determining the amount of urea present in carbamide peroxide formulations.

Carbamide peroxide is the active ingredient of over-the-counter oral antiseptics such as Cankaid™, Gly-Oxide™ and Debrox™, and is thought to form *in situ* from hydrogen peroxide and urea. Urea is known to decompose to ammonia and carbon dioxide or carbonate in the presence of an aqueous solution that contains an acid or a base [10]. Besides performing a more accurate analysis of urea contained in these products, this LC assay will provide insight into the stability and potency of the formulations. This method will also help in establishing specifications for the products as well as in determining the shelf-life of the products.

The stationary phase that was used in this study was a calcium bonded ion-exchange polymer (Interaction CHO-620) which is typically used for the separation of monosaccharides and sugar alcohols. Water is usually the eluent, however, in some cases organic modifiers are added. The column is typically heated in order to provide better peak shape and lower retention. The monosaccharides and sugar alcohols have been shown to be retained on the column via a ligand exchange mechanism [9, 11].

The variables that may have an effect on the urea separation were studied and the assay was optimized according to those results. The LC assay that was developed easily separates urea from any excipients that are present in the different carbamide peroxide formulations.

Effect of $CaNa_2$ EDTA concentration

The first variable studied was done to determine how the concentration of $CaNa_2$ EDTA in the mobile phase would affect the retention of urea. The retention of urea did not change appreciably as the mobile phase concentration of $CaNa_2EDTA$ was increased. However, the sensitivity of the system was found to be affected by the $CaNa_2EDTA$ concentration. As the concentration of $CaNa_2EDTA$ was increased, sensitivity decreased and baseline noise increased. This may be attributed to an increase in the absorption of UV light as the concentration of $CaNa_2EDTA$ present in the mobile phase is increased. Figure 1 shows how the peak area for the urea standards decreased with increasing mobile phase concentration of $CaNa_2EDTA$. The largest peak area for the urea standards was found to be at about 20 mg l^{-1} of $CaNa_2EDTA$. However, peak area did not decrease considerably until a concentration greater than 50 mg l^{-1} of $CaNa_2EDTA$ was used.

It was determined that if little or no $CaNa_2EDTA$ was added to the mobile phase, the urea peak shape was unacceptable. This study indicated that a mobile phase concentration of $CaNa_2EDTA$ between 25 and 50 mg l^{-1} provided acceptable results. Although the retention mechanism of urea is not known, it may be attributed to complexation between the urea, the stationary phase and $CaNa_2EDTA$.

Effect of column temperature

The column temperature has been shown to have a significant affect on analyte retention when using a carbohydrate column [12]. The effect of column temperature on urea retention was determined. It was found that retention decreased with increasing temperature (Fig. 2). Peak shape did not change significantly over the temperature range studied, however, temperatures below 60°C were not studied due to high back pressures. A temperature of 85°C was chosen since it provided good peak shape and a fast separation relative to lower temperatures. The high column temperature did not appear to adversely affect the stability of the column. The column provided acceptable separations and peak shapes even after several months of use.

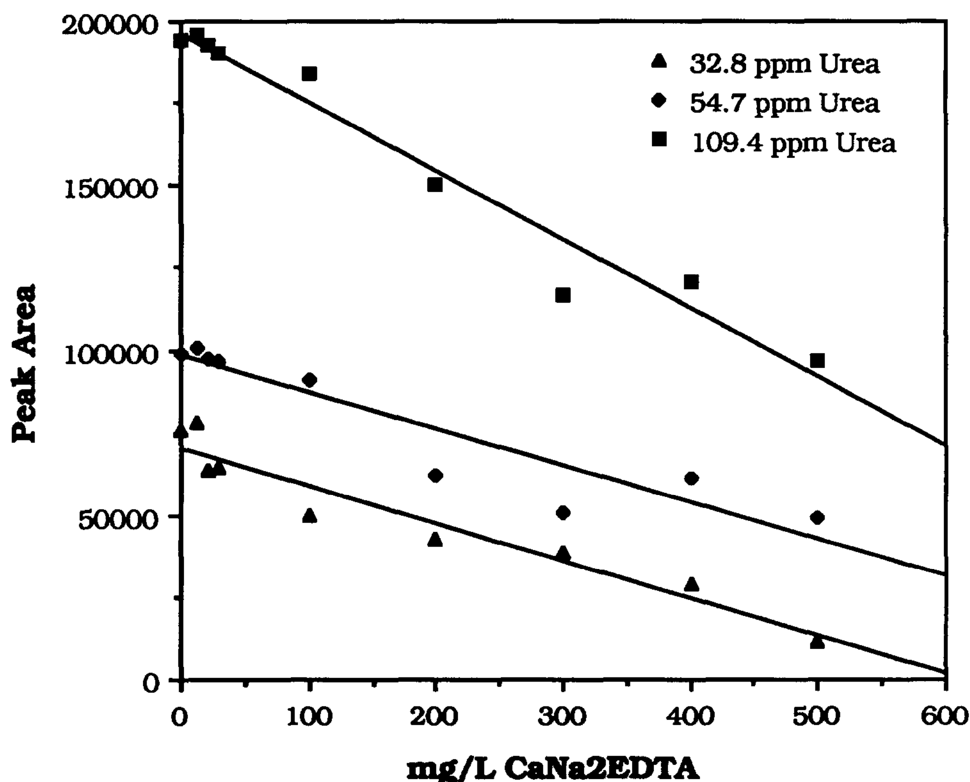

Figure 1
Effect of $CaNa_2EDTA$ on urea standards peak area. Mobile phase: $CaNa_2EDTA$ in water, column temperature: 85°C, flowrate: 0.6 ml min^{-1}.

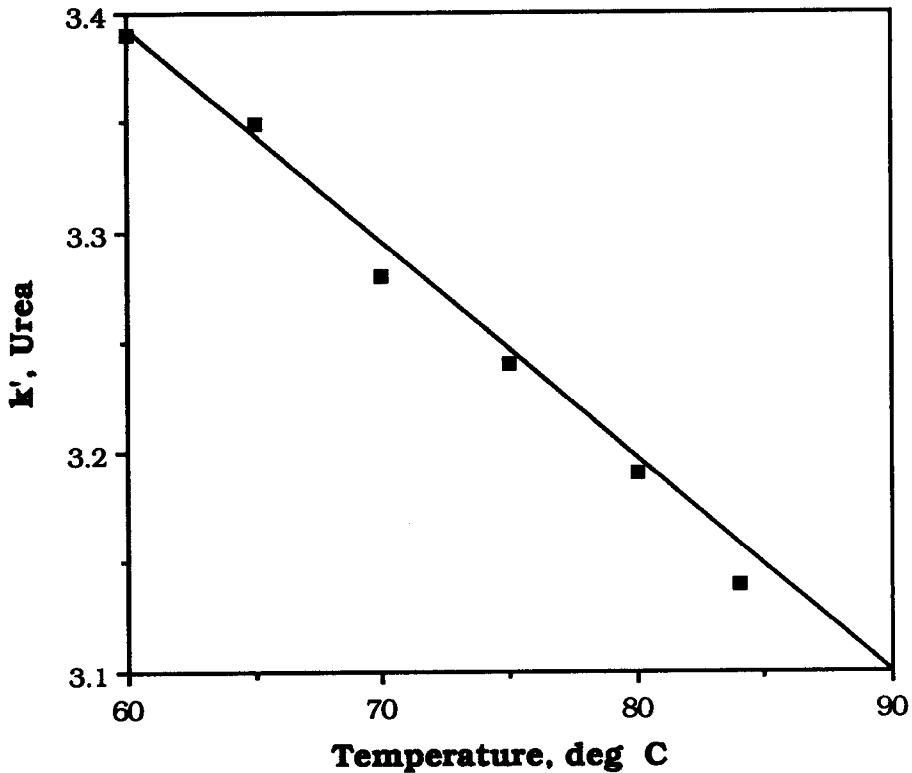

Figure 2
Effect of temperature on urea retention. Mobile phase: 50 mg ml^{-1} $CaNa_2EDTA$ in water, column temperature: variable °C, flowrate: 0.6 ml min^{-1}.

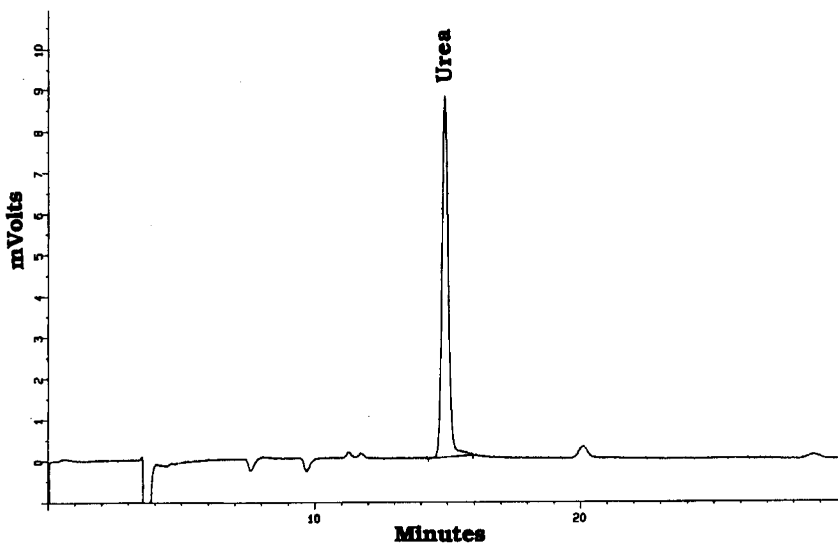

Figure 3
Typical chromatogram of a 103 μg/g urea standard. Mobile phase: 50 mg ml^{-1} $CaNa_2EDTA$ in water, column temperature: 85°C, flowrate: 0.6 ml min^{-1}, detection: UV, 210 nm.

Typical separations

Figures 3 and 4 show typical separations for a 98.1 μg/g urea standard and a carbamide peroxide sample, respectively. The excipients from the carbamide peroxide sample did not interfere with the urea peak. The urea peak shape was found to be acceptable with a run-time of less than 25 min.

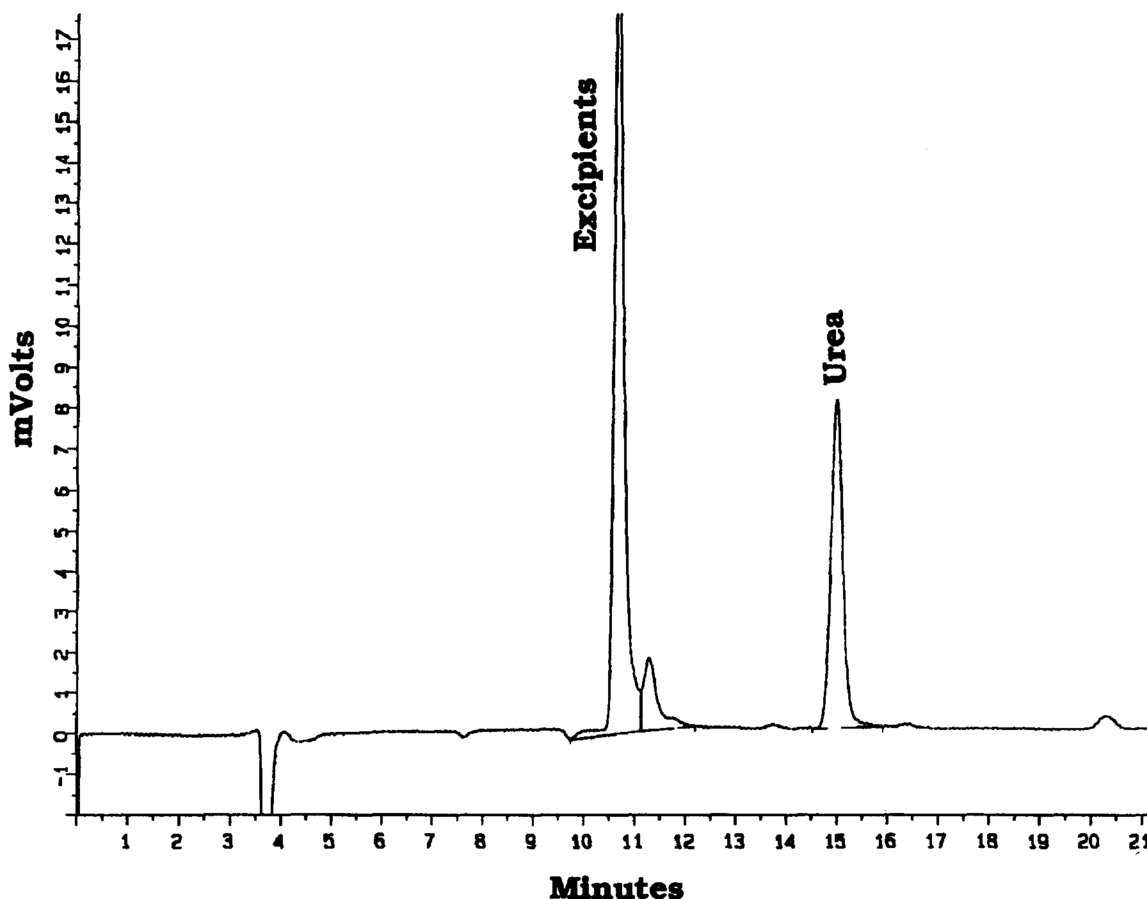

Figure 4
Typical chromatogram of a carbamide peroxide sample. Mobile phase: 50 mg ml^{-1} $CaNa_2EDTA$ in water, column temperature: 85°C, flowrate: 0.6 ml min^{-1}, detection: UV, 210 nm.

Calibration data

The linearity of the system was determined by analysing urea standards over the concentration range of 20 to 340 μg/g (duplicate injections). The system was found to be linear over this range with a correlation coefficient of 1.000, a *y*-intercept of 1.2197 and a slope of 0.8844.

The system precision was determined by injecting a known standard (103.5 μg/g) five times into the LC system and determining the reproducibility. It was found that the system precision had an %RSD of less than 1.0%. The method precision was determined by injecting carbamide peroxide samples (10 separately weighed samples) into the chromatographic system and determining the %RSD. The method precision was found to be less than 5.0%. Recoveries of 97.4% (1.0% RSD) and 98.3% (1.1% RSD) of the theoretical value of urea contained in two different carbamide peroxide formulations was obtained (Table 1). Triplicate injections were made for each sample. The carbamide peroxide samples used in this study contained approximately 6.6% urea on a weight to weight basis.

Table 1
Reproducibility data for two different carbamide peroxide formulations

Sample	% Urea found	% Urea found
1	98.1	97.8
1	99.1	98.9
1	98.6	98.6
2	98.8	98.6
2	97.3	98.2
2	97.8	98.9
3	96.6	98.6
3	95.9	98.9
3	96.3	98.1
4	97.2	96.6
4	97.2	96.1
4	97.0	96.4
5	96.6	99.7
5	97.0	99.4
5	97.8	99.4
Average	97.4% (1.0% RSD)	98.3% (1.1% RSD)

The data obtained in this study indicates that this chromatographic method may be used to determine the amount of urea present in carbamide peroxide samples.

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