Method development tools for the analysis of complex pharmaceutical samples

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The analysis of formulated drugs is a continual challenge due to the complexity of the samples. A single chromatogram in which all the components of such a complex mixture are represented by single peaks that are well resolved from each other would be ideal.

The complexity of samples entails differences in size, charge, hydrophobicity, and chemical variety of the components. Tablet formulations generally contain three different groups of chemicals: small organic molecules, polymers that are controlled-release agents and binding materials, and solid particulates. *Table 1* lists the most commonly used formulation materials, but space does not permit a listing of all possible components. The commercially available formulations comprise an astronomical variety of components and only a general categorization is possible here.

One of the difficulties of the analytical chemist is the discretion on the part of the suppliers. The excipient supplier companies use only a few basic polymers, such as polyethylene glycol (PEG), polyvinylpyrrolidone (PVP), hydroxypropyl methylcellulose (HPMC), and hydroxypropyl cellulose (HPC), to name a few. These simple names, however, cover a very wide range of molecular weights and hydrophobicity. The custom products are blends of these basic polymers developed for a specific tablet formulation

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and are usually mixed to a required viscosity. The information about the pharmaceutical polymers is limited to their trade names and viscosity. The chemical name of the component is occasionally available, but molecular weight distribution and component ratios are not. The alternative is to develop an analytical method to obtain the necessary information. The increasing numbers of chemical entities for drug development mandate the knowledge of the formulation components for accelerated troubleshooting of analytical issues such as drug substance (DS) recovery from formulations.

Poor recovery of drug substances can result from any one or a combination of three factors: DS can derivatize the polymer excipient(s), it can be entrapped in the polymer structure, and it can adsorb onto the solid formulation components.

Table 1

Representative list of commonly used tablet formulation excipients

Small organic molecules	Polymeric components	Solid components
Drug substance(s)	PVP	Croscarmellose
Lauryl sulfate salts	HPMC	Granulated cellulose
Magnesium stearate	HPC	Titanium oxide
Lactose	PEG	Silicon dioxide
Fatty acid and salts	Cellulose	Arabic gum
Citric acid	Starch glycolate	Microcrystalline cell
Inorganic salts	Cellulose acetate	
	Cellulose	
	Alginates	
	Glycerol	
	Pectin	

The separation of the drug substance component of a tablet, in most cases, is a straightforward scientific exercise. At the preformulation and final product analysis stage of drug development, the compounds are well known and a wealth of information is available for the method development chemist. The challenging part in formulation analysis is when recovery, i.e., "the case of the missing drug" is observed. The analytical chemist is faced with the problem of how to identify the formulation components and evaluate whether they are interfering with the drug substance recovery. The ultimate analysis is for the drug substance identification and quantitation, but with a very complex sample mixture, matching method development is necessary.

Method development difficulties present themselves in two major areas: the separation of the chemically diverse components and their detection. The use of reversed-phase liquid chromatography (RPLC) and multiple detection was evaluated for the analysis of tablet formulations.

RPLC in the gradient elution mode is the method of choice in cases in which small and polymer molecules can be separated in a single separation step. In gradient RPLC, the individual small and polymer molecules can be separated, in contrast to size exclusion chromatography (SEC), in which only group separation can be achieved.

The detection of such diverse compounds is a challenge of great magnitude. Most small organic molecules of pharmaceutical consequence are UV active. Many exhibit fluorescence as well. However, most of the cellulose-based formulation polymer macromolecules are stealth (transparent) to UV-VIS detection. Refractive index (RI), the most commonly used detection method for polymer molecules, is an effective way to visualize polymers. When mixtures contain small and polymer molecules, the gradient elution mode should be the method of choice. RI detection can be complicated when the gradient elution mode has to be used over a wide concentration range. The elevation in baseline signal with increasing organic solvent concentration is a hurdle that must be overcome for peak integration.

The evaporative light scattering detector (ELSD) is gaining popularity in pharmaceutical analysis. ELS can also be used for the analysis of small and polymeric molecules. Since tablet analysis in most cases is not signal sensitive, the benefit of good integration should override the detection sensitivity issue because of the flat baseline that occurs during gradient elution. The only limitation of ELS is that the solvents and salts used for the mobile phase have to be volatile, but the problem is not insurmountable.

The ultimate goal in tablet analysis is the separation and visualization of all components in a single chromatogram.

It should be emphasized that the mobile phase requirement for ELS is identical to that for mass spectrometry. The use of ELS for the method development of the LC portion of the LC-MS methods may allow more cost-effective use of the mass spectrometer. Separation methods can be developed on an LC-ELSD and then transferred and optimized for LC-MS. An ELSD is only a fraction of the cost of a mass spectrometer.

As mentioned above, the ultimate goal in tablet analysis is the separation and visualization of all components in a single chromatogram. RPLC in gradient elution (GR) mode using diode array or UV detection in combination with on-line ELS detection is well suited for the analysis of complex formulations. In order to complete the method development toolbox, an evaluation standard containing the major components of interest is also recommended.

Materials and methods

Chromatography

The HPLC system consisted of two SCL-10Avp pumps, SCL-10Avp system controller, SIL-10ADvp autosampler, SPD-10Avp UV-VIS detector (all from **Shimadzu,** Columbia, MD), and PL-ELS 1000 ELSD (**Polymer Laboratories,** Amherst, MA). For data acquisition, the CLASS-vp™, version 5.03, software system was used (**Shimadzu**).

The HPLC column used in this work was a silica-based reversed-phase analytical column with the following specifications: ZORBAX™ 300SB-C8, 4.6 × 150 mm, 5-µm spherical particles with 300-Å pores (**Agilent Technologies,** Wilmington, DE).

The standard mobile phase gradient was linear starting at 10% mobile phase B and ending at 90% mobile phase B over a period of 30 min. The mobile phase was maintained at 90% B for an additional 10 min and then returned to the starting conditions over a period of 5 min. Injection volume was 20 µL; detection wavelengths were 205 and 232 nm. The UV detector was followed in line by the ELS 1000. The ELSD operated with nitrogen gas flowing at 1.5 L/min; the nebulizer temperature was maintained at 95 °C and the evaporator temperature at 135 °C.

Reagents and materials

All solvents were of HPLC grade and were from **Fisher Scientific** (Pittsburgh, PA) unless otherwise stated. Binary gradient mobile phase A consisted of 0.01% trifluoroacetic acid (TFA) (**Aldrich,** St. Louis,

MO) in HPLC water; mobile phase B consisted of 0.01% TFA in 90% n-propanol and 10% HPLC water.

Opadry® samples were from **Colorcon** (West Point, PA). EVAX-005, an evaluation mixture standard (**iGORi,** Thousand Oaks, CA), contains a drug substance (DS1) as pseudoephedrine (PSEUD). The polymer components of the evaluation mixture were HPC, HPMC, PEG, and PVP. The drug substance of interest (DS2) was added to the evaluation mixture standard. The stock and working standards were stored at 2–8 °C and were stable for at least three months. The analytical samples were prepared by adding an appropriate amount of DS2 to EVAX-005. Sample solutions were filtered before chromatography with a 0.45-µm syringe filter.

Experimental

Pharmaceutical drug products present a significant challenge to the analytical chemist supporting formulation development and final product analysis. The samples are complex, and the methods should provide acceptable resolution and good detection. For this study, an EVAX-005 mixture prepared from traditional components of drug formulations was used.

The purpose of this paper is to emphasize the utility of the RPLC-GR-UV-ELSD system and EVAX-005. The methodology was developed as a tool with which to study drug–polymer interactions and the extraction of soluble components from tablet formulations.

In real tablet analysis, the components are extracted and the extraction solvent is analyzed for component content. Ideally, the components do not interact and the drug substance is quantitatively recovered. The difficulties related to extraction using the analytical method reported here will be described in a subsequent article.

Size exclusion chromatography, in which the polymer components are separated from the small molecules, is traditionally used for tablet analysis. The problem with this method, based on the authors' experience, is that it does not provide information as to drug substance-polymer interaction. Some of the components of the polymer matrix are UV active, and their signal overlaps the DS signal when DS eluted with the polymers because of polymer derivatization or entrapment. Accountability of the mass balance is skewed.

An approach in which all soluble components from the extraction solvent are visualized and separated was chosen. Consequently, mass balance for all participating components can be established. This method provides a deeper understanding and optimization of the sample preparation step. Comprehension of liquid extraction is a valuable aid in method troubleshooting.

The presence of components that are not relevant to the study can affect recovery, resolution, and reproducibility. Drug substance analysis is generally performed by a single isocratic method in which the drug substance and its possible degradation products are well separated. Sometimes it is difficult to obtain

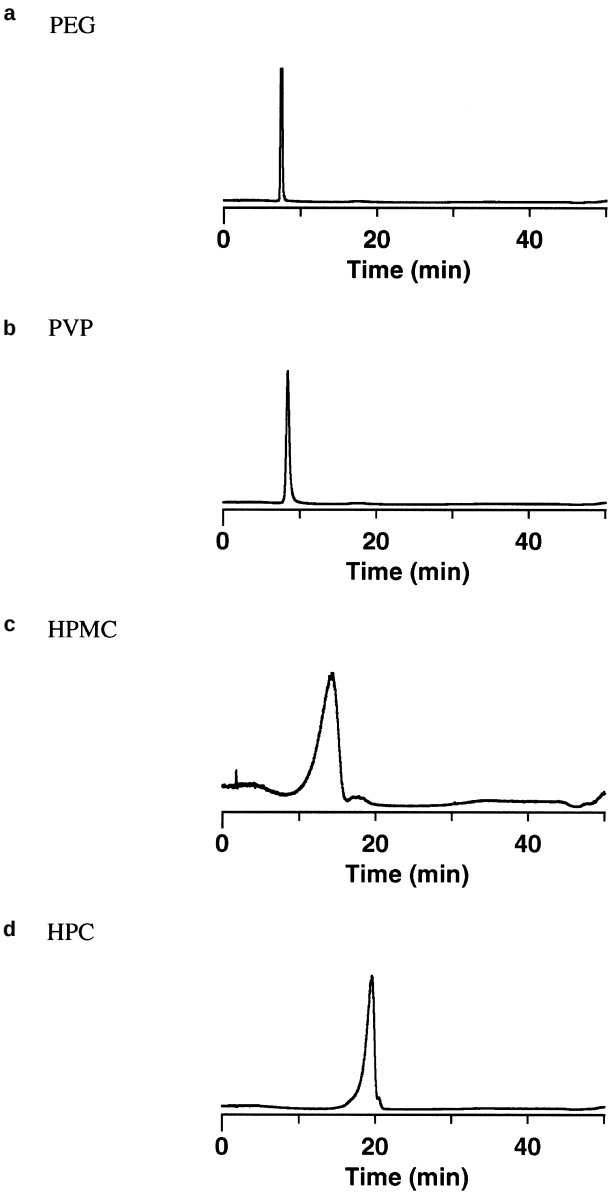

Figure 1 *Chromatograms of water-soluble polymers. Conditions—column: 300-Å pore size, silica based, C8, 4.6 × 150 mm; linear gradient elution: 10–90% B solvent; mobile phase A: 0.01 TFA in water, mobile phase B: 45% n-propanol and 0.01% TFA in water; ambient temperature: flow rate: 1 mL/min. a) PEG, b) PVP, c) HPMC, d) HPC.*

reproducibility of retention time or peak area. An understanding of the relationships between the drug substance, formulation polymers, and stationary phase can help to eliminate this problem. Formulation excipients can adsorb to the stationary phase. Polymer adsorption can alter the elution mechanism and influence retention time reproducibility.

In order to understand the phenomena involved, a single gradient elution system was developed in which all of the soluble tablet components were eluted and visualized. This method allows sample preparation

and resolution optimization ultimately helps to eliminate unwanted molecular interactions. The RPLC-GR-UV-ELSD method can assist in the understanding of the extraction procedure and retention discrepancies of the formulation components.

Reversed-phase chromatography

Reversed-phase chromatography has become the method of choice for the analysis of drug substances. Three important issues should be considered when working with polymers: size, solubility, and signal.

The hydrodynamic radius of linear water-soluble polymers in aqueous media is about an order of magnitude larger than that for a globular protein of the same molecular weight. A 300-Å pore size silica-based reversed-phase stationary phase was selected for this work. The role of pore size will be detailed in a later article.

For the reversed-phase chromatography of proteins, Cohen et al.[1] showed that acetonitrile (AcN)-based eluents are not appropriate for larger proteins. Recovery and peak shape problems were indicative of the solubility problem. The change to n-propanol-based eluents eliminated the solubility problem for hydrophobic proteins and good recovery was obtained. In preliminary experiments, similar signs were observed. AcN-based mobile phase, which is traditionally used in gradient elution, is not a suitable organic solvent for water-soluble polymers. n-Propanol as the organic modifier, on the other hand, results in acceptable separation and recovery.

With the exception of PVP, the polymers used in these experiments are not UV active. In gradient elution, RI detection is not feasible because of baseline drift. ELSD eliminates the baseline drift and provides a flat baseline in gradient elution, in addition to all soluble tablet components; small molecules and macromolecules can be seen with a single detection method.

Figure 1 displays the chromatograms of the individual polymer components used in this study. These components were selected because they are ingredients in virtually every tablet formulation.

Figure 1*a* shows the chromatogram of PEG. PEG products have a narrow molecular weight distribution and they elute as sharp peaks. PEG is not UV active.

Figure 1*b* shows the chromatogram of PVP. Due to its solubility and complex-forming character, PVP is widely used in drug formulations.[2] Its primary function is as a tablet binder. PVP is a hydrophilic polymer and consequently elutes early in the gradient system. PVP is UV active; the removal or at least the identification and separation from the main peak of interest are essential. The resolution between PEG and PVP is low. In this study, a simple gradient was preferred and the separation was not optimized. Since most of the problems originated with the HPMC, the PEG/PVP resolution was acceptable.

Figure 1*c* demonstrates the chromatography of HPMC. The most commonly used components of drug formulations are HPMC. The chemical name covers a very broad spectrum of products. HPMC formulations are available in a variety of molecular weight and derivatization levels. They are frequently supplied in various blends of the basic polymers. Most blends have to conform to certain viscosity requirements as the only measurable description. Because of the physical mixing, the products can represent a very complex mixture at the molecular level.

The molecular heterogeneity is also represented in a broad peak, even in gradient elution. The contribution of derivatization level and molecular weight to the peak shape requires further method development. However, more scientific exercise will lead to better control of the formulations, extraction,[3] and analytical method development. The fact that we can visualize and estimate their concentration is sufficient for this paper.

Figure 1*d* is a chromatogram of HPC. The elution at the end of the gradient indicates that HPC is the most hydrophobic polymer of all, and it is not UV active. Most drug substances elute before the HPMC, and some coelute with it. Because small molecules

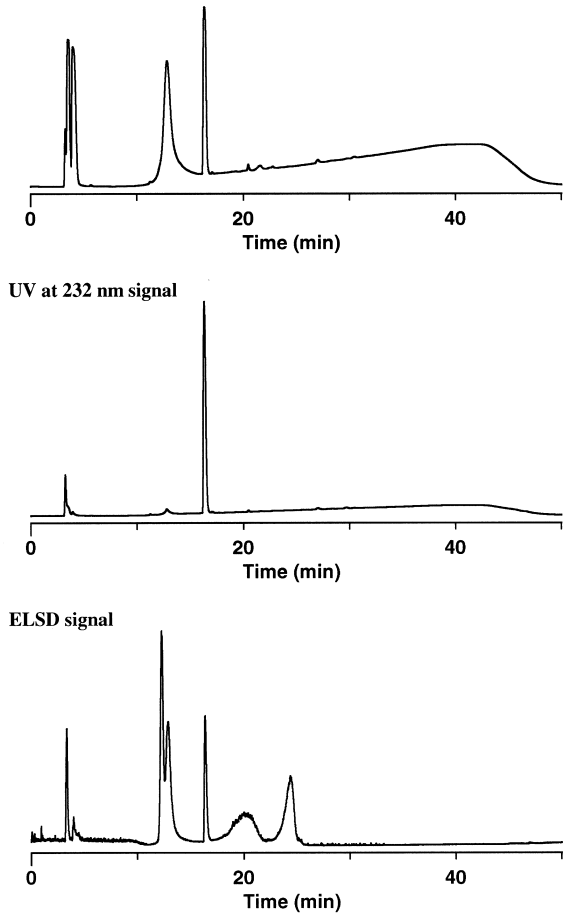

Figure 2 *Chromatogram of the evaluation mixture. Conditions as in Figure 1.*

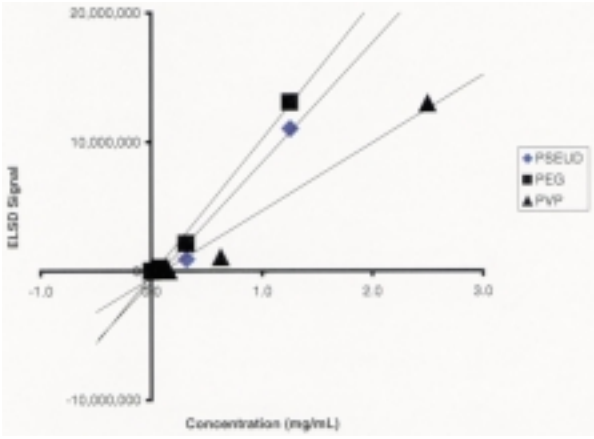

Figure 3 *Calibration curves of polymers using the ELS signal.*

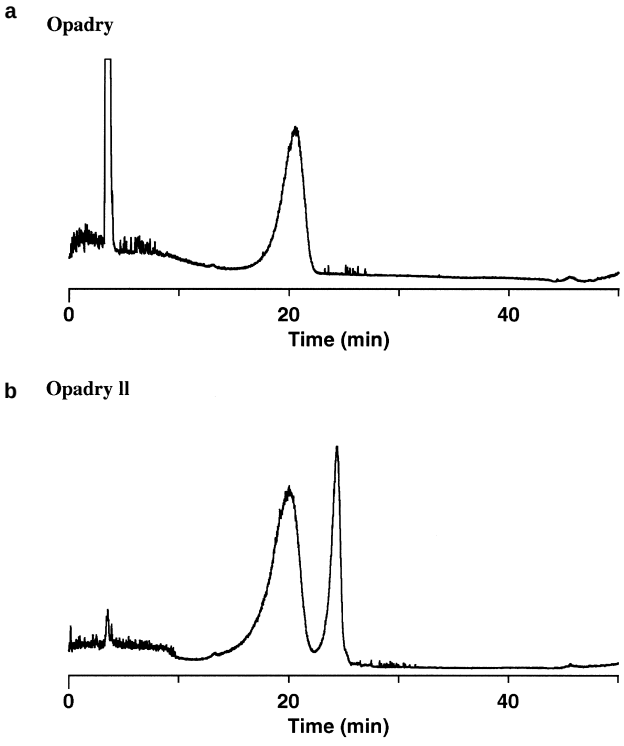

Figure 4 *Chromatograms of Opadry samples. Conditions as in Figure 1. a) Opadry, b) Opadry II.*

elute in a sharp peak as opposed to the broad HPMC peak, they are easily identifiable. Since most drug substances are UV active, they can be observed on the UV signal of the chromatogram.

Sample analysis

The sample mixture used in this work contained the EVAX-005 (DS1, PEG, PVP, HPMC, and HPC) and a second drug substance (DS2). A typical chromatogram is shown in *Figure 2*. Three signals were collected: UV at 205 and 232 nm and the ELS signal.

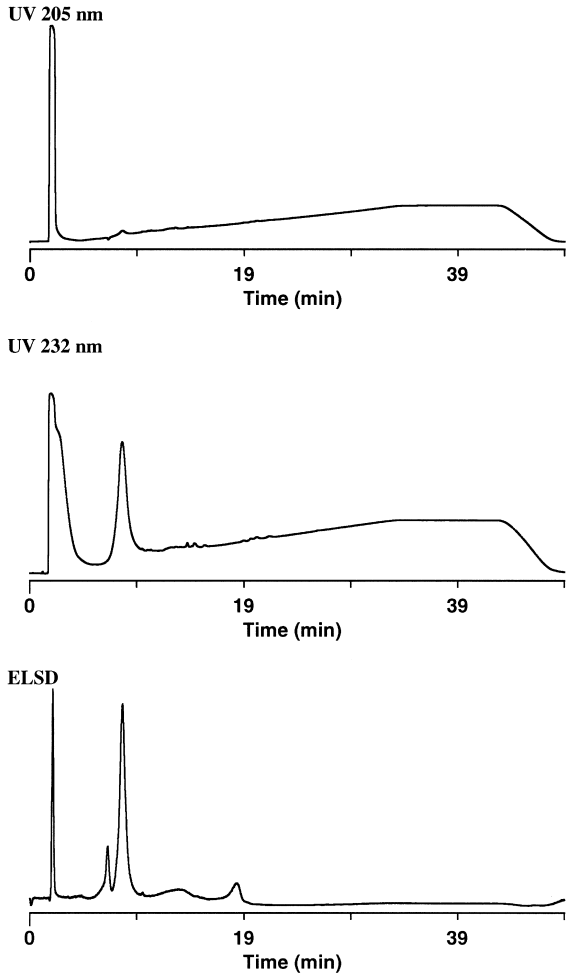

Figure 5 *Chromatogram of extracted tablets. Conditions as in Figure 1.*

The benefit of three signals is apparent: at 205 nm, DS1, DS2, and PVP (which elutes in a broad peak) can be detected. At 232 nm, PVP gives a small signal, but DS2 is still present as a strong peak. However, the ELS signal shows all of the components of the evaluation mixture.

Standard curves

Figure 3 displays the standard curves for some of the selected compounds. All standard curves show excellent linearity (r >0.9700). The major benefit of ELSD is that all of the different compounds can be analyzed quantitatively, even in a complex formulation like the evaluation mixture. The integration of HPMC and HPC is more difficult due to the heterogeneity of the sample.

Next, two Opadry samples were analyzed. Various Opadry products are widely used as film-coating systems for tablet coating.[4] The samples were extracted in water, centrifuged, and filtered prior to chromatography. *Figure 4a* is the RPLC chromatogram of

Opadry, and Figure 4*b* is that of Opadry II. It is clear that the common polymer component of the two samples is HPMC. The small shift in elution time for Opadry suggests that the HPMC is different than in the Opadry II sample. HPC gives a strong peak in Opadry II. A detailed study of Opadry may be possible when drug interaction is observed. However, the RPLC-GR-UV-ELS method provides sufficient information about these products for analytical method development. Size exclusion chromatography alone can assist in the molecular weight distribution of Opadry and other polymer mixtures. Unfortunately, most polymer blends are manufactured of the same or similar molecular weight components.

Tablet extraction

A placebo tablet formulation containing the components of the EVAX-005 was chosen for analysis. *Figure 5* displays the three signals of a chromatogram of the same tablet extraction. PEG, PVP, HPMC, and HPC can be identified from the chromatograms obtained at different detections. The profile of the chromatogram is similar to the standard extraction mixture, indicating that the tablet contains the same polymers; however, their relative concentration is different. The example indicates the utility of the method and the EVAX-005 standard.

Conclusion

The RPLC-GR-UV-ELSD method permits the analysis of complex pharmaceutical tablet preformulations and formulations. All components of the evaluation mixture were separated with a single reversed-phase gradient elution method. The benefits of evaporative light scattering detection were also demonstrated. The ability to visualize and quantitate all components of a complex sample while obtaining a flat baseline in gradient elution is an important goal in chromatography. The technique is generally applicable to drug substance extraction studies and optimization, dissolution, and preformulation studies.

The elution of the drug substance, relative to the polymer components, could have a significant effect on the recovery of the drug substance during extraction. When the drug substance elutes prior to the polymers, no extraction problems are expected. When the drug substance coelutes with the polymers, especially with HPMC, recovery and reproducibility issues may emerge. Since the elution order of compounds is usually proportional to their hydrophobicity, the elution order of the components can also be considered as an experimental hydrophobicity scale. This information can be very useful in designing solid- and liquid-phase extraction conditions.

The elution of the drug substance relative to the polymer components is also an indication of the efficacy of the controlled-release application. Drugs, which elute before HPMC, are probably not good candidates for hydrophobicity-based controlled release formulation. Compounds, which are more hydrophobic than HPMC, might interact too strongly with the polymer, Drugs that coelute with the polymers may exhibit reproducibility problems.

In comparison to the DS, the polymers are in excess, and can adsorb to the hydrophobic surface. Polymer adsorption generates a new, more hydrophilic surface and the elution time changes accordingly. The use of gradient elution eliminates some of these problems, since the surface is regenerated by the gradient.

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4. Information on film coating products can be found at www.colorcon.com.

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