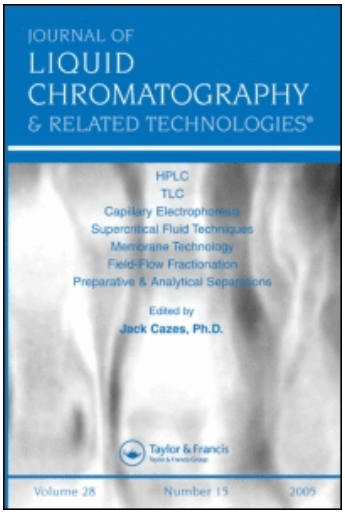

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A Rapid HPLC Assay for the Determination of Dextro-propoxyphene Related Substances in Combination with Aspirin, Acetaminophen, and Caffeine in Tablet and Capsule Formulations

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A Rapid HPLC Assay for the Determination of Dextro-propoxyphene Related Substances in Combination with Aspirin, Acetaminophen, and Caffeine in Tablet and Capsule Formulations

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ABSTRACT

A 23 min gradient HPLC assay has been developed to simultaneously measure related substances of dextro-propoxyphene salts (napsalate, hydrochloride) and other actives that are routinely found in combination in capsules or tablets: aspirin, acetaminophen, and caffeine. This method has shown the ability to resolve all known active ingredients, impurities, and degradation products from the propoxyphene and other active drug parent peaks. All the propoxyphene related substances are quantified against a 1-point propoxyphene external standard prepared at 2% of the typical sample concentration. Propoxyphene related substance measurements occur at 210 nm. For acetaminophen combinations,

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wavelength switching is used starting at 280 nm to measure acetaminophen related substances, then switching to 210 nm for propoxyphene related substance measurements. Two sample solvents can be used for propoxyphene related substance quantitation, the second of which is used to provide enhanced room temperature stability-about one month-for aspirin containing formulations. This method provides a simple, one-step solution for related substance analysis rather than numerous separate assay methods for each active's related substances.

Key Words: HPLC; Propoxyphene; Acetaminophen; Aspirin; Caffeine; Salicyclic acid; Validation.

INTRODUCTION

Dextro-propoxyphene, as either hydrochloride or napsalate salt forms, is a widely prescribed analgesic alone, or in combination with other drugs: aspirin, caffeine, or acetaminophen. Official USP-NF analytical methods for the propoxyphene, alone or in combination, include labor-intensive sample extractions for GC analysis or various HPLC methods. The known related substances of propoxyphene-acetoxy analog and carbinol hydrochloride-utilize the labor intensive GC assay. Aspirin and caffeine combinations utilize two separate assay techniques: the labor-intensive GC assay for caffeine and a colorimetric assay for degraded aspirin to salicylic acid. Aspirin related, substance-free, salicylic utilizes the colorimetric test. Acetaminophen related substances, 4-aminophenol and 4-chloroacetanailide, are determined by UV or TLC, respectively.[1]

A reverse phase HPLC method has been published for potency analysis of the dextropropoxyphene napsalate, caffeine, aspirin, and salicylic acid.[2] However, that method utilized samples prepared in methanol which is known to accelerate degradation of aspirin in solution.[3–14] Though that HPLC method claimed seven day stability of aspirin in methanolic solution, this is contrary to our experience and the experience of other Refs.[3–14] that indicate rapid deterioration to salicylic acid in less than 24 hours. No method has been found to simultaneously measure related substances of all the active drug components in a single method.

A 23 min reverse phase gradient HPLC method on C8 columns has been developed that provides quantitative related substance determination for propoxyphene alone or in combination with other drugs. Related substance determinations for the other active drugs can be determined concurrently. Two sample solvents are used: one for aspirin containing combinations that provide about one month room temperature stability for the aspirin, and a second for all other samples or combinations. Propoxyphene is stable in both sample

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Table 1. Reagents and chemicals.

Chemical names	Source
Acetaminophen, aspirin, caffeine, dextro-propoxyphene salts, salicylic acid, and propoxyphene related substances (acetoxy analog and carbinol hydrochloride)	Corporate reference standards
4-Aminophenol	Aldrich Chemical, Milwaukee, WI
4-Chloroacetanalide	Aldrich Chemical, Milwaukee, WI
Hydroquinone	Aldrich Chemical, Milwaukee, WI
Acetylsalicylsalicylic acid	Acros Organics, NJ
Salicylsalicylic acid	Acros Organics, NJ
Hydrochloric acid, 0.02 N (prepared from conc. reagent)	Fisher, FairLawn, NJ
Potassium phosphate monobasic, HPLC grade	Fisher, FairLawn, NJ
Water, HPLC grade	Fisher, FairLawn, NJ
Acetonitrile, HPLC grade	Fisher, FairLawn, NJ
O-Phosphoric acid, HPLC grade	Fisher, FairLawn, NJ
Formic acid, GR	EM Science, Gibbstown, NJ
Citric acid monohydrate	Mallinckrodt, Paris, KY

solvents for at least seven days at room temperature; however, acetaminophen solutions must be stored at 5°C to achieve four day stability per ICH Guidelines on stability.[15]

EXPERIMENTAL

Reagents and Chemicals (Table 1)

Sample Solution Preparation

Primary sample solution: 60% hydrochloric acid (0.02 N)/40% acetonitrile. Alternate sample solution (for aspirin stability): 99 parts acetonitrile, 1 part formic acid, and 0.1 part citric acid.

Mobile Phase Preparation

Mobile phase A—a 50 mM solution of potassium phosphate monobasic was prepared in purified water. The solution was adjusted to pH 2.4 ± 0.1

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using phosphoric acid. Mobile phase B—60% acetonitrile/40% mobile phase A.

Instrumentation and Chromatographic Conditions

A HPLC system comprised of a Waters 2690 Separations Module (Alliance System), Waters 2487 variable UV detector, a PE Nelson box interface, and a Hewlett Packard HP1000 data system was used for all experiments. Two alternate Agilent HPLC columns were used, 5 μm particle, 4.6 × 150 mm; a Zorbax SB C8 or an Eclipse XDB C8. The following gradient program was applied (Table 2).

Initially, all analysis was done at 210 nm to maximize sensitivity. An alternate wavelength switching program was adopted for analysis of acetaminophen and its related substances starting at 280 nm, then switching back to 210 nm for analysis of other active drugs related substances.

Since analysis is being done at 210 nm for most of the analytes, extreme care must be taken to ensure that glassware is clean and reagents are HPLC grade or better. Any contamination can affect the baseline quality, especially later in the gradient where we wish to quantify very small propoxyphene related substances. Routinely during our development studies, we incurred significant baseline shifts due to glassware. The corrective actions were to remove mobile phases, methanol wash and thoroughly dry the mobile phase flasks, methanol wash the HPLC column to remove all contaminants, then remake fresh mobile phases and check baselines. This whole process is the hardest part of this low UV assay, but controllable with care.

Table 2. Gradient conditions: linear gradient from A to B.

Time	Flow	%A	%B
0.0	1.0	85	15
12.0	1.0	25	75
13.0	1.0	0	100
18.0	1.0	0	100
18.5	2.0	85	15
22.0	2.0	85	15
22.1	1.0	85	15
23.0	1.0	85	15

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Preparation of Standard Solutions

Separate propoxyphene standards are prepared for each salt form being analyzed. Approximately 0.5 mg/mL solutions as the propoxyphene base were prepared for each salt form (for HCl salt, MW = 375.98, prepare about 50–55 mg propoxyphene hydrochloride per 100 mL in primary sample diluent; for napsalate salt, MW = 564.48, prepare about 90–100 mg propoxyphene napsalate per 100 mL). A 1/50 dilution is made of each propoxyphene stock solution in the same sample solvent to achieve 2% standards for quantitation of propoxyphene related substances.

Separate stock standards for acetaminophen and caffeine were prepared in primary sample solvent and diluted to achieve about 1% standards for quantitation of their related substances. Separate 1 mg/mL aspirin and salicylic acid stock standards are prepared in the alternate sample solvent and diluted 1/100 to quantify aspirin related substances. (Note: propoxyphene standards can also be prepared in the alternate sample solvent, but caffeine and acetaminophen chromatography peak distortion prevents use of this solution use for those standards).

Preparation of Standard Curves

Separate calibration curves were made for each active drug and their known related substances to determine response factors of all components relative to propoxyphene, and to determine the limit of quantitation (LOQ)/limit of detection (LOD) of propoxyphene. A propoxyphene hydrochloride standard solution in primary sample solvent was diluted across a concentration range of 0.8–40 mcg/mL (0.16–8% typical sample concentrations of propoxyphene and bracketing the routine 2% standard used for 1-point quantitation). Similar ranges of other active drugs and their individual related substances (except aspirin and salicylic) were prepared in primary sample solvent. For aspirin and salicylic comparisons to propoxyphene, all stock solutions including propoxyphene and standard curves were made in alternate sample solvent.

Preparation of Capsule and Tablet Formulations for Analysis

Four different formulations manufactured by Eli Lilly and Co. were prepared in sample solvents (aspirin containing tablet prepared in both solvents using the alternate sample solvent to quantify both aspirin and propoxyphene related substances, then the primary sample solvent to again quantify propoxyphene related substances along with caffeine). Related

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Table 3. Formulations tested.

	Ingredient levels (mg)			
Active drug	Capsule #1	Capsule #2	Tablet #1	Tablet #2
Propoxyphene	65.00	65.65	101.00	50.50
Acetaminophen	None	None	None	325.00
Caffeine	None	32.72	None	None
Aspirin	None	389.00	None	None

substance quantitation of propoxyphene in both solvents was compared within method precision studies to ensure no statistical difference in results occurred. The ingredient compositions given in Table 3 were tested.

Preparation of Placebo Samples

Excipient ingredients in each tablet or capsule formulation were prepared, without the active drugs, in sample solvents to simulate levels expected in the 0.5 mg/mL propoxyphene base equivalent sample preparations. Each placebo was profiled against its respective active drug sample preparation to assess interferences. Placebo mixtures were also exposed to heat stressing (80°C for three and seven days) to determine if any impurities due to the placebos could be generated that would interfere with analysis of active drug related substances. Additionally, each color ingredient and other tablet coating ingredients were evaluated for interferences.

RESULTS AND DISCUSSION

Our United Kingdom affiliate had established a potency method for acetaminophen and propoxyphene combined in one tablet formulation that used wavelength switching from 280 nm for acetaminophen analysis to 205 nm for propoxyphene. That method utilized a 4.6 mm × 25 cm Kromasil C18 with 40% acetonitrile in a 12 millimolar, pH 2.5 potassium phosphate buffer at 1 mL/min isocratic analysis. Though propoxyphene related substances (acetoxy and carbinol compounds) and acetaminophen related substances (4-aminophenol and 4-chloroacetanailde) are resolved from the active drugs in this method, separate methods are used to analyze those compounds. The acetoxy and carbinol are analyzed using a perchloric acid buffer at pH 2 on a C18 column and 217 nm UV detection, while 4-aminophenol is analyzed

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Table 4. Typical retention times of active ingredients, related substances, impurities, and degradation products in propoxyphene formulations.

Peak identity	Typical retention time (sec)
Void time	60
Capsule #2 placebo peak	85
4-Aminophenol, degradation of acetaminophen, starting material in synthesis	95
Formic acid, sample diluent component	112
Acetic acid, aspirin degradation	119
Hydroquinone-solution degradation peak of aminophenol and acetaminophen	202
Acetaminophen	252
Caffeine	347
Orange color mixture for tablet coating	376
Napsalate peak of propoxyphene salt	419
Aspirin	526
Salicylic acid	578
Carbinol derivative of propoxyphene	668
4-Chloroacetanalide, synthesis int. of acetaminophen	690
Acetoxy derivative of propoxyphene	711
Propoxyphene	775
Acetylsalicylsalicylic acid, aspirin deg. Late peak #1	849
Salicylsalicylic acid, aspirin deg. Late peak #2	905
Late aspirin deg. Peaks 3–8	949–1150

using butane sulfonate ion-pairing with a C18 column and 272 nm UV detection. The 4-chloroacetanalide in acetaminophen is tested using a thin layer chromatography method.

We were interested in developing a single method to be used for the determination of related substances in each of the four propoxyphene formulations. The conditions above, for propoxyphene and acetaminophen potency gave poor chromatography of aspirin and salicylic, insufficient retention of the 4-aminophenol, and incomplete elution of some of the aspirin degradation analogs. Shorter carbon chain reverse phase columns and gradient optimization was pursued to get the fastest run time possible, while providing good chromatography of all the active compounds and their related substances. Both methanol and acetonitrile in pH 2.5 potassium phosphate gradients were evaluated on various C8 columns at 210 nm, a wavelength where we appeared to have similar response of propoxyphene and its related substances, while providing good sensitivity for other target compounds.

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Table 5. Relative response factors.

Compound	Response factor vs. propoxyphene
Response at 210 nm	
Acetoxy	1.1
Carbinol	1.1
Acetaminophen	1.1
Caffeine	2.5
Aspirin	0.9
Salicylic acid	3.6
4-Aminophenol	0.9
4-Chloroacetanalide	1.0
Response at 280 nm	
Acetaminophen	0.27
4-Aminophenol	0.20
Caffeine	0.66[a]

[a]Response is 2.45 × acetaminophen, similar to 210 nm.

Ultimately, a 10 µL sample injection onto 15 cm Zorbax SB C8 and XDB C8 columns with an acetonitrile/phosphate buffer gradient was chosen for fastest and best chromatography of target compounds.

Selectivity

Table 4 summarizes typical retention times of active drugs, known related substances, degradation products, excipients, and tablet coatings.

The chromatograms given in Figs. 1 and 2 profile routine formulations and some heat stressed samples to force impurities for selectivity confirmation. These analyses are all done at 210 nm, only, without using wavelength switching for acetaminophen related substances.

Aspirin standards or a formulation containing aspirin was heat degraded at 80°C for three and seven days to promote the generation of salicylic acid and other aspirin degradation peaks in Fig. 2. This heat degraded sample is used as part of system suitability to confirm resolution of aspirin analogs from the propoxyphene peak and to assess resolution of the aspirin–salicylic acid critical pair.

Solution pH studies show that the critical pair resolution is best below a pH of 3 in typical reverse phase systems.[16] We evaluated the impact of low

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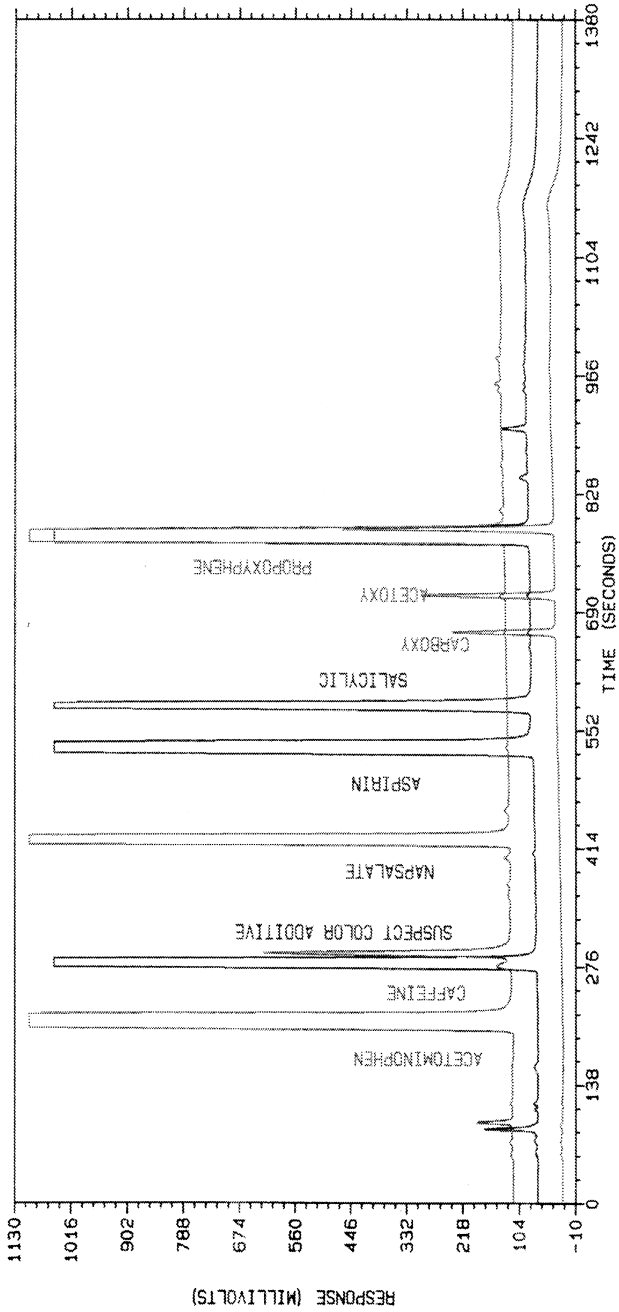

Figure 1. A typical selectivity profile.

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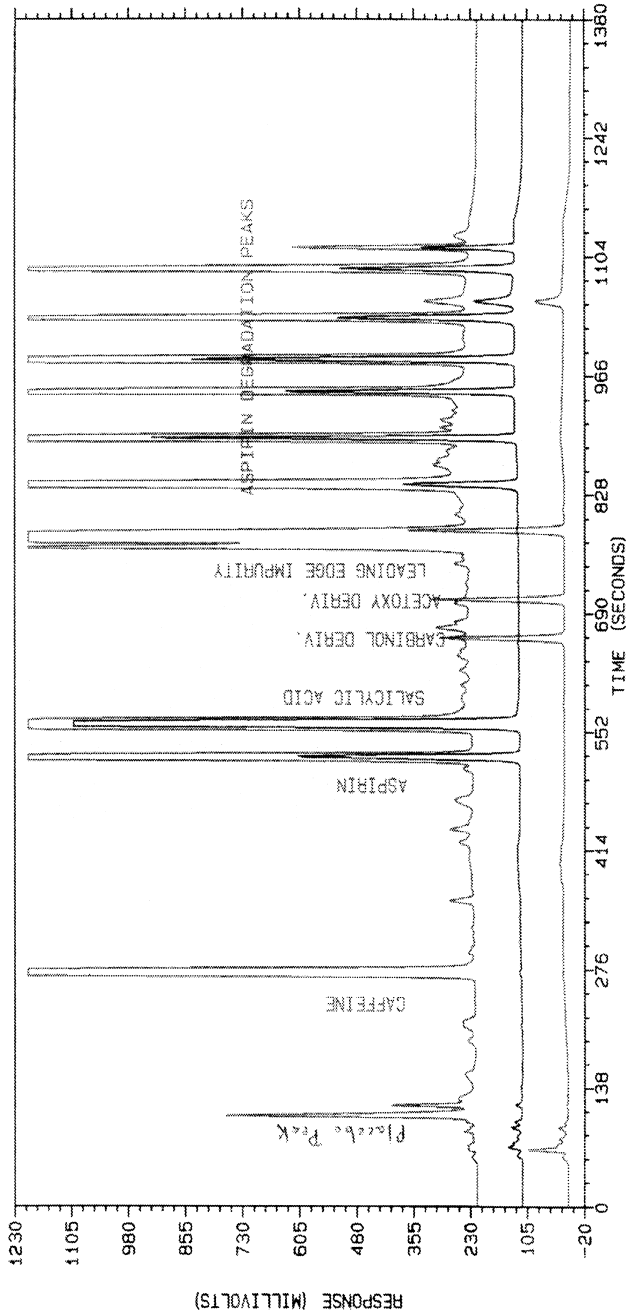

Figure 2. A typical selectivity profile.

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pH range (about 2–3) on all actives, impurities, degradation products, and excipient components. Only the aspirin–salicylic acid pair was affected with improved resolution as pH was lowered. We locked in on a pH of 2.4 ± 0.1 for routine analysis to maximize resolution without broaching the pH limit of the analytical columns at pH 2.

Linearity

Response factors of the active drugs and their known related substances were determined at 210 nm relative to propoxyphene using a ratio of curve slopes against the propoxyphene curve. Also, response factors for acetaminophen, aminophenol, and caffeine were determined at 280 nm (Table 5).

The LOD and LOQ were determined for propoxyphene hydrochloride using two separate techniques: $3 \times$ noise estimate from typical chromatogram = LOD and $10 \times$ noise = LOQ; alternatively, LOQ determined from the standard curve = $10 \times$ STD deviation of curve/mean response. Both estimates of LOQ provided similar results of 0.01% for sample preparations of 0.5 mg/mL, well below ICH Guidelines recommendation of 0.1% LOQ based on daily dose of drug.

For the typical 0.5 mg/mL propoxyphene sample preparations, the LOQ of free salicylic in the formulations is about 20 fold more sensitive due to the approximate 7 : 1 ratio of aspirin to propoxyphene in capsules tested and the 3+ response factor for salicylic acid. This method is very sensitive for free salicylic determinations.

Stability

Sample solution stability of all the active drugs and their known related substances were checked in primary sample solvent at room temperature. A change of less than 0.1–0.2% total related substances in the active drugs was accepted as an indicator of solution stability. All four propoxyphene formulations, propoxyphene and caffeine standard solutions were stable beyond seven days at room temperature.

Aspirin is not stable in the aqueous solvent, but in the alternate sample solvent aspirin sample solutions were stable at room temperature for 3–4 weeks. Salicylic acid begins forming almost immediately when aspirin is dissolved in primary sample solvent.

Acetaminophen in primary sample solvent degrades to aminophenol, which then degrades to hydroquinone at room temperature. However, if samples are stored refrigerated at 5°C, stability is achievable for up to four days. The hydroquinone degradant was determined by GC/MS in aged aminophenol solutions and confirmed with spiking experiments. This

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degradation pathway appears to be unique. An organic text investigated for aminophenol in the presence of HCl and acetonitrile only suggested the Pinner synthesis (alcoholysis of nitriles using conc. HCl) to produce carbamides.[17] We only use 0.02 N HCl solution with acetonitrile in the primary sample solution and room temperature aging.

Caffeine generated no related substances when stored for weeks in solution. Additionally, when caffeine was heat stressed at 80°C for three and seven days and put in solution, no related substances were detected.

Accuracy

Placebo samples of each formulation were prepared and wet spiked with standard solutions to assess recovery. At five times typical placebo ingredient ratio to propoxyphene levels, 99–101% recovery was achieved except with the formulation containing a clay product-Kaolin. Quantitative recovery (>95%) was not achievable until Kaolin to propoxyphene ratio neared a 2 : 1 typical mix. One hundred percent was recovered in the typical formulation mix.

Precision

Both intermediate precision and repeatability experiments were done using all formulations. Additionally, precision of propoxyphene was checked using the alternate sample diluent to compare to primary sample diluent for capsule #2 containing aspirin (Table 6).

CONCLUSION

This simple, 23 min HPLC method provides a quick, quantitative determination of propoxyphene related substances in solid dose formulations with stability and limits of quantitation that exceed ICH Guidelines. Additionally, this method can be used to determine related substances of other active drugs formulated in combination with the propoxyphene concurrently with the

Table 6. Precision estimates of propoxyphene related substance measurements.

	Capsule #1-diluent 1	Capsule #2-diluent 1	Capsule #2-diluent 2	Tablet #1	Tablet #2
Repeatability	3.13%	5.50%	1.66%	3.78%	1.71%
Int. precision	3.60%	5.67%	4.43%	2.75%	5.11%

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propoxyphene. Enhanced stability of aspirin, up to one month at room temperature, allows for accurate quantitation of free salicylic acid formed over time in aged formulations.

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