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HPLC method development for AG-85 and benzyl alcohol in anhydrous topical formulations

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Summary

AG-85, a lipophilic drug synthesized by rational drug design, is inhibitory to cellular growth in vitro by inhibition of the enzyme thymidylate synthase which catalyses the rate-limiting step in the de novo synthesis of thymidine nucleotides (Appelt et al., *J. Med. Chem.*, 34 (1991) 1925–1934). A pharmaceutically elegant anhydrous topical formulation of AG-85 was developed for treatment of psoriasis (Pavliv et al., *Int. J. Pharm.*, (1994) in press). Benzyl alcohol was used as a preservative in the topical formulation. HPLC methods were developed and validated to quantitate AG-85, AG-85 impurities, and benzyl alcohol. Precision, accuracy, limit of quantitation (for Category II quantitative), selectivity, linearity range, and ruggedness were the analytical parameters addressed for these methods. The validated methods meet current GMP requirements.

Introduction

Validation of new analytical methods of analysis for pharmaceutical products is a requirement of Current Good Manufacturing Practice (cGMP) regulations, [21 CFR 211.194(a)]. Accuracy and reliability of test methods must be demonstrated before use to determine compliance of pharmaceutical products with established specifications. Suitability of test methods must be verified under actual conditions of use in accordance with cGMP requirements. Analytical methods have different validation requirements depending upon the specific use of the assay. The USP defines three categories of assays. Category I assays are methods for quantitating the major components of bulk drugs or active ingredients in drug products, including preservatives. Category II assays are methods for quantitating impurities and/or degradation products, in bulk drugs and drug products. Two types of limits tests are included: limit of quantitation for low concentration of impurities, degradation products, etc.; and limit of detection to determine the lowest level of analyte detectable in a sample. Category III assays are analytical methods for evaluating performance characteristics (USP XXII, 1990).

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Precision, accuracy, selectivity, linearity range, and ruggedness are the analytical parameters which require validation for Category I assays. Category II assays require validation of limit of quantitation or limit of detection, depending on usage, in addition to those parameters required for Category I assays. A Category III assay requires validation for precision and ruggedness, and any of the other parameters that may be applicable for that particular method (USP XXII, 1990).

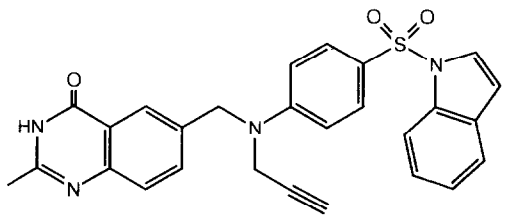
The analytical method for AG-85 and AG-85 impurities was validated for Categories I and II (quantitative only) assays, respectively, according to the USP. The analytical method for benzyl alcohol was validated as a USP Category I assay. The limits of detection (Category II, Limit Tests) were not addressed by either method as these methods were not intended for trace analyses (USP XXII, 1990).

Anhydrous topical formulations of AG-85 were developed for treatment of psoriasis. The first stage of manufacturing requires AG-85 to be dissolved in solubilizers, prior to addition of the semisolid ingredients. This is referred to as the in-process solution. HPLC methods using a Gilson HPLC equipped with a UV detector and autosampler were developed and validated to assay in-process solutions for AG-85 and final product for AG-85 at 1.25% and benzyl alcohol at 2.0%. The method for AG-85 was also developed to separate and quantitate the synthetic intermediates, I5 and I6, which could be present as impurities in the drug product, since they have been detected in bulk drug lots. A multi-step isocratic elution method and a single step isocratic method were developed and validated to quantitate AG-85 and AG-85 impurities, and benzyl alcohol, respectively, in anhydrous topical formulations.

Materials and Methods

Materials

The bulk chemical, AG-85, *N*-(N^4-(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl)-N^4-(prop-2-ynyl)sulfanilyl)indole, is manufactured by Agouron Pharmaceuticals, Inc. The structure of



AG-85

Fig. 1. Structure of AG-85.

AG-85 is shown in Fig. 1. The drug product, a topical cream containing 1.25% AG-85, was developed at Agouron Pharmaceuticals, Inc. Multiple lots of bulk drug with varying impurity profiles and final drug product were used for development and validation of the assay methods.

Inactive excipients consisted of saturated polyglycolyzed glycerides (Labrafil® M-2130CS), PEG 8 caprylic/capric triglycerides (Labrasol®), diethylene glycol monoethyl ether (Transcutol®), glyceryl behenate (Compritol® 888 ATO), all from Gattefosse Corp., Westwood, NJ, glyceryl monostearate and polyoxyethylene stearate (Arlacel® 165) from ICI Americas, Inc., Wilmington, DE, lauryl lactate (Ceraphyl® 31) from Van Dyk & Co., Inc., Belleville, NJ, benzyl alcohol NF, and butylated hydroxytoluene FCC (BHT), from Spectrum Chemical Mfg Corp., Gardena, CA (Pavliv et al., 1994). Formulations with varying concentrations of AG-85 and benzyl alcohol were prepared for the validation studies, along with placebos to demonstrate no interference of excipients with the methods.

Acetonitrile, methanol, ammonium acetate, and water were HPLC grade (Fisher Scientific, Fair Lawn, NJ). Potassium phosphate monobasic, sodium phosphate dibasic buffer salt, pH 7.41, was Fisher certified. Buffer solutions were filtered and degassed with FP-Vericel PVDF 0.45 μm filters (Gelman Sciences, Ann Arbor, MI). Acetonitrile and methanol were degassed by sonication.

Gelman nylon Acrodisc 13, 0.45 μm syringe filters and Whatman nylon, 0.45 μm Uniprep

filters (Whatman, Inc., Clifton, NJ) were used interchangeably for filtering the sample solutions.

HPLC conditions

A Gilson HPLC equipped with a variable UV detector, two pumps, dynamic mixer, dilutor, autosampler, and 20 μl sample loop, was used as the primary instrument. Detector sensitivity was set at 0.2 absorbance units full scale (AUFS) with an injection volume of 100 μl. (A 100 μl injection volume was used to overfill the 20 μl sample loop to ensure adequate flushing.) Peak width was set at 1.00 min with a minimum area of 100. Column temperature was not controlled.

The following conditions are suggested parameters. Baseline separation of the critical band pair, AG-85 intermediates, I5 and I6, is the determining factor for optimizing instrument conditions. Retention time may vary depending on mobile phase preparation and column efficiency.

AG-85 and AG-85 impurities A reverse phase CN column (Zorbax, 250 × 4.6 mm, 5 μm) was used, with a reverse phase CN guard column (Zorbax, 12.5 × 4.0 mm, 5 μm). The detector was set at 300 nm, with a 0.5% peak sensitivity. A multistep isocratic elution was used with an initial composition of 45% acetonitrile, 55% 0.1 M ammonium acetate; for 9.50 min; then ramp up to 60% acetonitrile in 0.20 min; 60% acetonitrile, 40% 0.1 M ammonium acetate from 9.70 to 17.00 min; then ramp up to 100% acetonitrile in 0.20 min; 100% acetonitrile for 17.20 to 20.00 min; ramp down to 45% acetonitrile in 0.20 min; 45% acetonitrile, 55% 0.1 M ammonium acetate from 20.20 to 25.00 min total run time with a 2.0 ml/min flow rate.

Benzyl alcohol A reverse phase C8 column (Zorbax, 250 × 4.6 mm, 5 μm) was used, with a reverse phase C8 guard column (Zorbax, 12.5 × 4.0 mm, 5 μm). The detector was set at 220 nm with a 2.0% peak sensitivity. The mobile phase was isocratic; 67% methanol, 33% 0.01 M phosphate buffer with a 1.0 ml/min flow rate and 20 min run time.

Standards preparation

AG-85 stock solution, approx. 200 μg/ml 20 mg of AG-85 were accurately weighed to the nearest 0.01 mg into a 100 ml volumetric flask, methanol added to volume and sonicated 15–30 min to facilitate dissolution.

Benzyl alcohol stock solution, approx. 400 μg/ml 0.4 g of benzyl alcohol were accurately weighed to the nearest 0.1 mg into a 100 ml volumetric flask and methanol added to volume. 10 ml were pipetted to a 100 ml flask and diluted to volume with methanol.

Standard dilutions Serial dilutions were prepared by pipetting 20, 15, 10 and 5 ml of the AG-85 stock solution and 20, 15, 10 and 5 ml of the benzyl alcohol stock solution into 25 ml volumetric flasks and diluting to volume with methanol.

Sample preparation

Solutions Three replicates were prepared for each sample. About 1 ml of AG-85 topical formulation in-process solution was filtered with a 0.45 μm nylon filter; 0.25 g of filtrate was accurately weighed to the nearest 0.1 mg into a 50 ml volumetric flask and methanol added to volume.

Topical creams Three replicates were prepared for each sample. Approx. 0.9–1 g of AG-85 topical formulation was weighed to the nearest 0.1 mg into a 100 ml volumetric flask. The flask was filled to about 1–2 ml below mark with methanol and placed in a 45–50°C shaker bath at medium-high speed overnight (12–16 h), cooled to room temperature; sonicated for about 10 min, cooled to room temperature, and diluted to volume with methanol. The extract was then filtered with a 0.45 μm nylon filter and analyzed.

Multiple HPLC systems

The two methods were run on the following HPLC systems by a combination of laboratories and analysts to demonstrate precision, accuracy, ruggedness and suitability.

System I	Gilson	Method HPLC system
System II	HP 1090 M	Intralaboratory HPLC system
System III	Shimadzu	Interlaboratory HPLC system

In-process solutions In-process solutions were tested with the AG-85 method to recover AG-85

from solutions prepared at three concentrations of AG-85 and assayed by three analysts using three different HPLC systems (see Table 1).

Topical creams Topical creams were formulated at three concentrations of AG-85 and benzyl alcohol, extracted and assayed by three analysts using HPLC systems I and III (see Table 2).

Filter validation

AG-85 Solutions of AG-85 were prepared at two concentrations: approx. 50 and 2 μg/ml, in three different solvents: acetonitrile, methanol, and 60% PEG 400 in water. 3 ml of each solution were filtered once through a Gelman nylon Acrodisc 13, 0.45 μm syringe filter. 2 ml each of the filtrates were filtered four more times for a total of five filterings, utilizing a new filter for each filtration. 1 ml of air was pushed through each filter following the solution, to minimize the amount of solution lost in the filter at each filtration. Samples of each solution, unfiltered, filtered once, and filtered five times were analyzed by HPLC.

Benzyl alcohol Five benzyl alcohol standard solutions were prepared ranging from 85 to 429 μg/ml in methanol. A 3 ml aliquot of each standard solution was filtered once. 2 ml each of the filtrates were filtered four more times for a total of five filtrations. Samples of each standard solution were placed in a shaker bath overnight at 43–45°C. Samples of each standard, unfiltered, filtered once, filtered five times, and heated overnight with shaking (unfiltered) were analyzed by HPLC.

Assay

Each set of standards was injected from lowest to highest concentration. Samples were bracketed between sets of standards.

Calculations

The coefficients for the linear equation $y = mx + b$ were calculated using the linear regression least-squares method for the five standards where y is the peak area and x denotes the concentration in μg/ml of AG-85 or benzyl alcohol. Acceptance criteria of r^2 not less than 0.985 and the relative standard deviations (RSDs) of the standards not to exceed 4.00% were followed.

The concentration x for each sample was calculated using the calculated values for m and b and the AG-85 or benzyl alcohol peak area for y:

$$x_{\mu g\ AG\text{-}85/ml} = \frac{y_{\text{Peak area}_{\text{sample}}} - b}{m}$$

The percent AG-85 or benzyl alcohol was calculated using the following equation:

$$\%\ AG\text{-}85 = \frac{(\mu g\ AG\text{-}85/ml)(\text{Volume}_{ml})}{(g_{\text{sample}})(10^6\ \mu g/g)} \times 100$$

The area percent for each impurity was calculated for each sample cream/standard analyzed. The average area percent was calculated for each impurity for each sample cream/standard where replicate samples were prepared and tested. The average total impurities were calculated for each

TABLE 1

In-process solution AG-85 assay results [a]

AG-85 sample ID	Analyst	System [b]	Theoretical weight (%)	Avg. Calc. [c] weight (%)	SD
356.05.A	A	I	2.50	2.46 [e]	NA
	A	II	2.50	2.45 [e]	NA
334.86.B	A	I	2.50	2.44	0.04
334.86.B	B	III	2.50	2.48 [e]	NA
P-07-08202 in-process solution	A	III	2.50	2.44	0.01
334.128.D	C	I	2.50	2.41	0.02
334.128.E [d]	C	I	2.00	1.92	0.01
334.128.F [d]	C	I	1.50	1.43	0.01

[a] Data below condensed for purposes of publication.

[b] System I Gilson Method HPLC system
System II HP 1090 M Intralaboratory HPLC system
System III Shimadzu Interlaboratory HPLC system

[c] Averages are $n = 3$ starting from sample preparation of a single in-process solution; except where indicated by [e], then $n = 1$.

[d] These solutions were prepared as dilutions of solution 334.128D.

TABLE 2

Topical cream assay results [a]

Sample ID	Ref. [b]	Analyst	System	AG-85			Benzyl alcohol		
				Theoretical weight (%) [c]	Avg. Calc. weight (%) [d]	SD	Theoretical weight (%) [c]	Avg. Calc. weight (%) [d]	SD
356.06.A	1	A	I	1.23	1.22	0.02	2.00	2.12	0.02
356.06.B	1	A	I	1.23	1.22	0.01	2.00	2.05	0.16
356.06.C	1	A	I	1.23	1.24	0.01	2.00	2.12	0.01
334.86A	2	A	I	1.22	1.19	0.05	2.00	2.04	0.05
334.86.A [repeat]	2	A	I	1.22	1.21	0.01	–	–	–
P-02-06042		B	III	1.24	1.20	0.01	2.00	2.04	0.03
334.87	2	A	I	1.22	1.23	0.01	2.00	1.95	0.04
334.88	2	A	I	1.22	1.25	0.00	2.00	2.09	0.01
334.89	2	A	I	1.22	1.25	0.01	2.00	2.08	0.03
334.89 [repeat]	2	A	I	1.22	1.23	0.01	–	–	–
334.90	3	A	I	–	–	–	2.00	1.99	0.04
P-01-06032		B	III	–	–	–	2.00	1.95	0.03
P-06-08192		B	III	–	–	–	2.00	2.07	0.01
334.103	4	A	I	–	–	–	2.00	2.20	0.17
334.105	4	A	I	–	–	–	2.00	2.03	0.06
P-07-08202		B	III	1.22	1.20	0.01	–	–	–
334.104	5	A	I	1.22	1.24	0.01	2.00	2.06	0.04
334.106	5	A	I	1.22	1.26	0.01	2.00	2.08	0.02
334.108	5	A	I	1.22	1.26	0.01	2.00	2.08	0.02
334.129.A		C	I	1.21	1.22	0.00	2.00	2.12	0.01
334.129.B		C	I	0.96	0.97	0.03	2.40	2.55	0.04
334.129.C		C	I	0.71	0.71	0.01	1.60	1.57	0.02

[a] Data condensed for purposes of publication.

[b] (Ref. 1) All from same parent bulk batch, 356.06, filled into three different tube types. (Ref. 2) All from same parent bulk batch, P-02-06042, filled into three different tube types and bulk jars. (Ref. 3) From bulk batch, P-01-06032, filled into tubes. (Ref. 4) All from same parent bulk batch, P-06-08192, filled into two different tube types. (Ref. 5) All from same parent bulk batch, P-07-08202, filled into three different tube types.

[c] The theoretical weight percent of the cream is based on the average calculated weight percent of the respective in-process solution.

[d] Averages are of triplicate analyses starting from sample preparation of a single cream sample.

sample cream/standard by adding up the average area percents for each impurity peak.

Results and Discussion

Wavelength

AG-85 AG-85 has a primary λ_{max} at 225 nm, with a secondary λ_{max} at 300 nm. The λ_{max} at 300 nm was selected for quantitation, as there would be less interference from the excipients, mobile phase and noise than at 225 nm (see Fig. 2).

Benzyl alcohol Benzyl alcohol has a λ_{max} near 210 nm, but the UV cutoff of methanol is about 220 nm, which was the selected wavelength.

Column selection

AG-85 The Zorbax CN column is the column selected for AG-85 and AG-85 impurities. Intermediates I5 and I6 are the critical band pair. Separation can be achieved with a C18 column, but the peaks are quite broad. Use of the more polar CN column gives better peak shape. Adequate resolution is accomplished by varying the

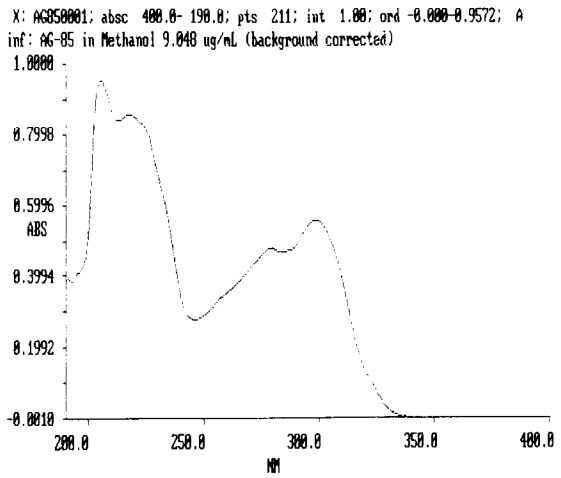


Fig. 2. AG-85 UV spectrum (9.048 μg/ml).

mobile phase composition and the flow rate. A mobile phase composition of 45% acetonitrile:55% 0.1 M ammonium acetate at 2.0 ml/min gave acceptable peak shape of the main AG-85 peak with a resolution, R_s, of about 1.5 for I5 and I6 such that:

$$R_s = \frac{(t_{I6} - t_{I5})}{(t_{wI5} + t_{wI6})}$$

where t_{I5} and t_{I6} are the retention times for peaks I5 and I6 less the dead volume time (t_0), and t_{wI5} and t_{wI6} denote the peak widths of I5 and I6 (Snyder et al., 1979).

Repeated analyses of the bulk drug showed a broad impurity peak at a retention time of about

```
Sample ID: METHANOL BLANK

Loop: 1/10
Peak width: 1.00 min
Peak sensitivity: 0.5%
Data Collection time: 24.70 min
Minimum area: 100

----Area percent report----
Peak Name                RT        Area      Area%
                      18.778       10788     29.866
                      22.076       20361     56.364
                      22.835        4974     13.770
  3 Peaks integrated
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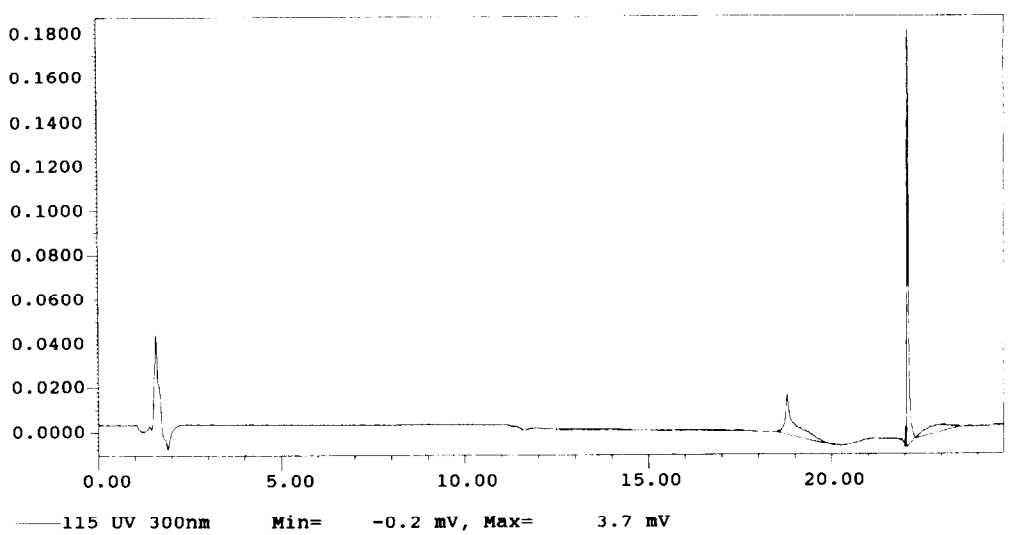

Fig. 3. Chromatogram of methanol blank showing responses due to mobile phase changes at 300 nm using the multi-step isocratic method on the Gilson HPLC system.

20–22 min that was inconsistently integrated. Increasing the acetonitrile to 60% after 9.5 min sharpens this impurity peak and shortens the elution time to 13.1 min, as well as a previously undetected impurity peak now eluting at 15.8 min, so that both can be reproducibly integrated (see Fig. 3, methanol blank chromatogram and Fig. 4, AG-85 chromatogram).

Benzyl alcohol The C8 column was selected based on the National Formulary method for analyzing benzyl alcohol for benzaldehyde (NF XVII, 1990). A chromatogram of benzyl alcohol along with other ingredients from the cream extract is shown in Fig. 5.

Guard columns Multiple analyses of cream samples deteriorate the columns, causing a shortening of retention times and increased tailing with some peak splitting of the main peak on the CN column. The C8 column would give broad peaks and an unstable baseline. Use of guard columns of the same column material helped eliminate these effects. Guard columns must be

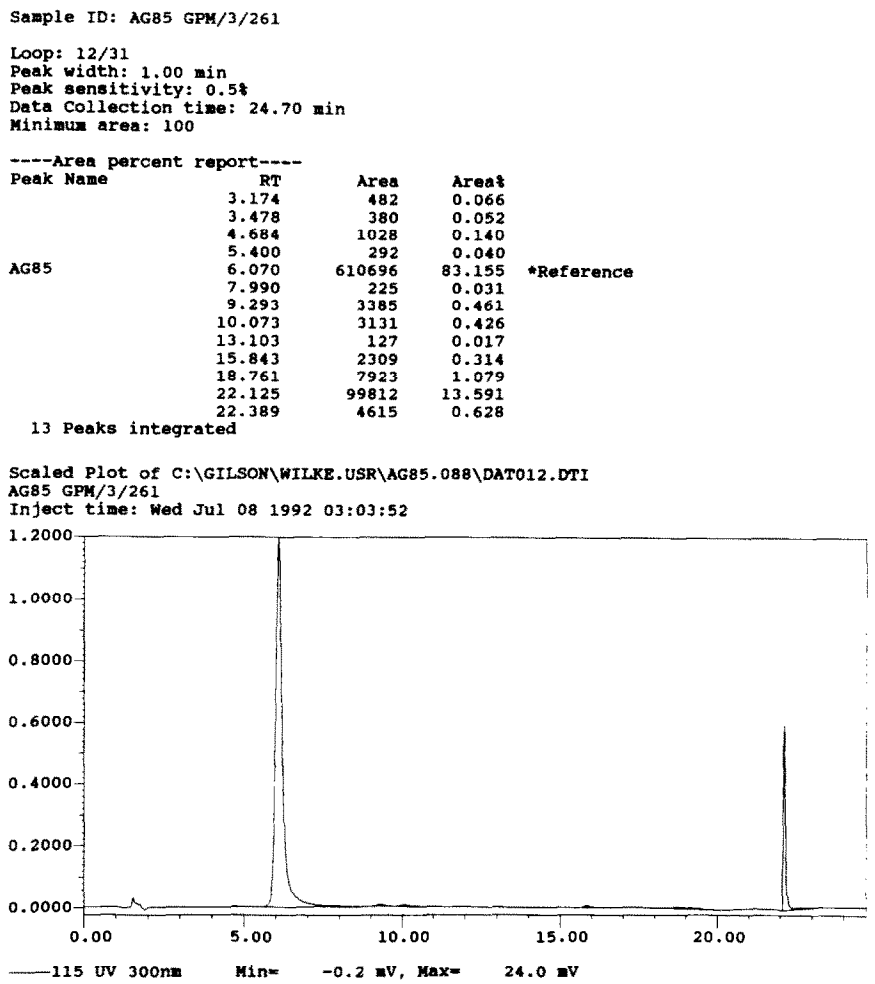

Fig. 4. Chromatogram of AG-85 in methanol at 300 nm, using the multi-step isocratic method on the Gilson HPLC system, showing separation of I5 and I6 at 9.39 and 10.26 min, respectively.

replaced frequently prior to saturation with the excipients, to protect the analytical column (Snyder et al., 1988).

Integration parameters

AG-85 The detector and peak sensitivities were varied to determine the optimum settings for the concentrations in the range of about 40–200 μg/ml of AG-85 in methanol. It was found that a detector sensitivity of 0.2 AUFS gives the most consistent integration of the AG-85 peak over the specified concentration range, as is demonstrated by a coefficient of determination, r^2, of greater than 0.985. The peak sensitivity of 0.5% maximizes the number of reproducibly detectable impurity peaks that can be detected at these concentrations. A minimum area count of 100 eliminates false peaks due to noise.

Benzyl alcohol The detector sensitivity at 0.2 AUFS and peak sensitivity at 2.0% gives consistent integration of the main peak over the specified concentration range, as is shown by a coefficient of determination, r^2, of greater than 0.985.

Linearity range

AG-85 Five standard solutions containing concentrations of AG-85 between 40 and 200 μg/ml were assayed five times in ascending order of concentration. Nine topical cream samples were assayed between each set of five standard

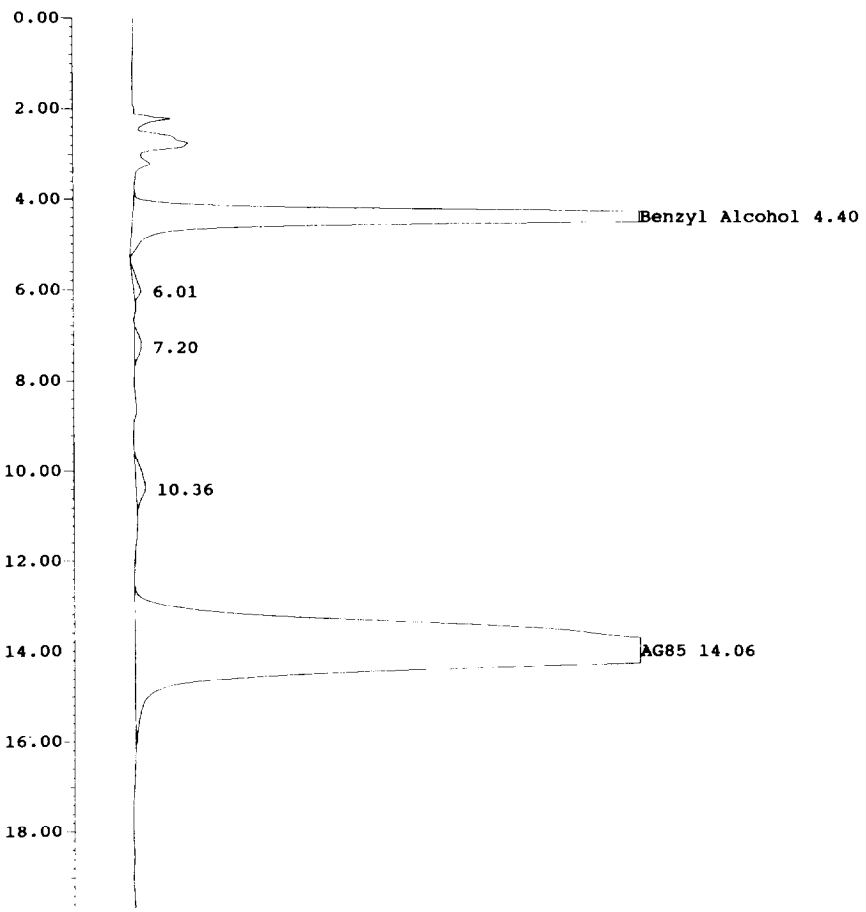

Fig. 5. Chromatogram of benzyl alcohol in topical cream extract at 220 nm using the single-step isocratic method on the Gilson HPLC system.

solutions to emulate actual test conditions. The parameters of linear regression for peak area vs concentration of the standard solutions were calculated. The linearity of the method was determined by simple least-squares analysis of data. The coefficient of determination, r^2, was calculated as 0.996. RSDs ranging from 2.18 to 3.69% for the various concentrations demonstrate acceptable reproducibility. The linearity range of AG-85 has been validated by verifying that standards at the extremes of the 40–200 μg/ml concentration levels give acceptable results in terms of accuracy and precision.

Benzyl alcohol Five standard solutions containing concentrations of benzyl alcohol between 75 and 400 μg/ml were assayed five times in order of ascending concentration. Nine topical cream samples were assayed between each set of five standards to emulate actual test conditions. The parameters of linear regression for the standard solutions were calculated. The linearity of the method was determined by simple least-squares analysis of the data. The coefficient of determination, r^2, was calculated as 0.994. RSDs ranging from 0.84 to 2.27% for the various concentrations demonstrate acceptable reproducibility across the range. The linearity range of benzyl alcohol has been validated by verifying that standards at the extremes of the 140–420 μg/ml concentration levels give acceptable results in terms of accuracy and precision. However, linearity below the 140 μg/ml will require further optimization of conditions.

Precision

AG-85 Precision was determined by reviewing data from standards injected on three different days, using three different CN columns. Nine topical cream samples were assayed between each set of standard solutions. The linear regression analyses of the HPLC assay for AG-85 standard solutions using three different CN columns gave r^2 values of 0.996, 0.997, and 0.999. The percent relative standard deviations (%RSD) on three different days for each AG-85 concentration ranged from 0.59 to 3.69.

Benzyl alcohol Precision was determined by reviewing data from five sets of standards at five concentration levels assayed on three different days, using three different C8 columns. Nine topical cream samples were analyzed between each set of standards. The linear regression analyses of the HPLC assay for benzyl alcohol standard solutions using three different C8 columns gave r^2 values of 0.994, 0.988, and 0.995. The %RSDs on three different days for the benzyl alcohol standards ranged from 0.75 to 5.08. Reanalysis of the linear regression of the data with an r^2 value of 0.988 (and unacceptable RSDs above 4.00%) by bracketing the first three, second three and third three sets of standards gave r^2 values of 0.995, 0.995, and 0.992 with RSDs ranging from 0.52 to 3.90 in the 140–420 μg/ml range, and met the acceptance criterion of RSDs not exceeding 4.00%.

Accuracy

AG-85 Accuracy of the method, including the extremes of the assay range, was determined by comparing one set of standards with a second set of standards on three days using three CN columns. The percent difference of theoretical value vs assay value at the extremes of the assay range was −0.73 to 1.92% for 40 μg/ml; and −0.30 to 0.65% for 200 μg/ml.

Benzyl alcohol Accuracy of the method, including the extremes of the assay range, was determined by comparing one set of standards with a second set of standards on three days using three C8 columns. The percent difference of theoretical value vs assay value was −8.08 to −10.63% at 75 μg/ml concentration. Standards at 140 μg/ml assayed from −1.57 to −2.97% difference from theoretical value; and 420 μg/ml standards assayed from −0.68 to −1.16% difference from theorectical value. This method is not accurate below 140 μg/ml.

Ruggedness

AG-85 Ruggedness of the method was previously demonstrated by comparing two series of standards tested five times on three different days with three different CN columns. Samples were run between each set of standards. Ruggedness of this method was further demonstrated by showing the results of the linear regression analy-

sis from an outside laboratory using a Shimadzu system and a fourth CN column, also assaying samples between each set of standards. The sample results are reported in Tables 1 and 2, run on System III. The linear regression analysis of the assay (outside laboratory) for AG-85 standard solutions gave an r^2 value of 1.000 with a percent relative range for each AG-85 concentration ranging from 0.19 to 0.65.

Benzyl alcohol Ruggedness of the method was previously demonstrated by comparing three series of standards tested five times on three different days with three different C8 columns. Nine topical cream sample solutions were assayed between each set of standards. Based on consistent results over three days the method is demonstrated to be rugged.

Limits of quantitiation of impurities

AG-85 Quantitation of impurities for AG-85 concentrations below 80 μg/ml would require a change of the detector sensitivity setting and further validation. The average area percent of AG-85 ranged from 98.17 to 98.64% with standard deviations ranging from 0.08 to 0.30 for the various AG-85 concentrations above 80 μg/ml over the 3 day period.

Selectivity

AG-85: In-process solutions Selectivity was demonstrated at three concentrations of AG-85 assayed by three different analysts utilizing three different HPLC systems. The AG-85 concentrations assayed at 95.3–98.4% recovery of the theoretical concentrations. The AG-85 in-process solution values were consistently lower than the theoretical weight percent. The theoretical weight percent is used to calculate the actual batch weight and is not adjusted to compensate for any losses or degradation that occurs during the arduous batch processing. The in-process solution data for AG-85 are summarized in Table 1.

AG-85: Topical creams Creams were extracted and assayed by three different analysts utilizing two different HPLC systems. The AG-85 concentrations assayed at 96.8–103.3% recovery of the theoretical concentrations. Recovery within $\pm$3% of theoretical is considered acceptable considering the extraction process required to recover AG-85 from the cream formulations. The topical cream data for AG-85 are summarized in Table 2.

Benzyl alcohol Creams were extracted and assayed by three different analysts utilizing two different HPLC systems. The benzyl alcohol concentrations assayed at 97.5–110% recovery of the theoretical concentration. The benzyl alcohol values determined tended to be slightly higher than the theoretical values. The theoretical weight percent is used to calculate the actual batch weight and is not adjusted to compensate for material losses during batch processing prior to the addition of benzyl alcohol. The topical cream data for benzyl alcohol are summarized in Table 2.

Filter validation

AG-85 The filtration study was performed to show that AG-85 does not bind to the nylon filters used to filter the cream extracts. Binding of AG-85 to the nylon filter was negligible.

Benzyl alcohol A similar study was performed to show that benzyl alcohol does not bind to the nylon filters and is stable during the extraction process for the cream formulations. There was no loss of benzyl alcohol due to binding on the filters, or loss or decomposition during the simulated extraction process.

Extraction from creams

AG-85 Heating the creams in acetonitrile or methanol to 45–50°C and shaking overnight leads to an AG-85 recovery of 98–99%. However, AG-85 in acetonitrile shows degradation within 2 weeks under 5°C storage conditions, whereas AG-85 in methanol is stable for at least 4 weeks at 5°C (data not shown). No AG-85 interfering peak was detected for placebo creams extracted under these same conditions. Spike recoveries from placebo creams were not performed, as AG-85 must be dissolved in a solubilizer prior to cream formulation. However, creams were formulated at AG-85 concentrations of 0.71, 0.96, and 1.21% with AG-85 recovery of 100.0, 101.0 and 100.8%, respectively.

Benzyl alcohol Initial extraction studies of benzyl alcohol from the cream formulations showed complete recovery. No interfering peaks were detected for creams prepared without benzyl alcohol.

Conclusions

AG-85 The HPLC method was validated for assaying the concentration of AG-85 and AG-85 impurities in the topical formulations according to the USP requirements for Category I and II quantitative assays (USP XXII, 1990).

Benzyl alcohol The HPLC method was validated for assaying benzyl alcohol concentration in the topical formulations as per the USP requirements for Category I assays (USP XXII, 1990).

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References

Appelt, K., Bacquet, R.J., Bartlett, C.A., Booth, C.L.J., Freer, S.T., Fuhry, M.A.M., Gehring, M.R., Herrmann, S.M., Howland, E.F., Janson, C.A., Jones, T.R., Kan, C.C., Kathardekar, V., Lewis, K.K., Marzoni, G.P., Matthews, D.A., Mohr, C., Moomaw, E.W., Morse, C.A., Oatley, S.J., Ogden, R.C., Reddy, M.R., Reich, S.H., Schoettlin, W.S., Smith, W.W., Varney, M.D., Villafranca, J.E., Ward, R.W., Webber, S., Webber, S.E., Welsh, K.M., and White, J., Design of enzyme inhibitors using iterative protein crystallographic analysis. *J. Med. Chem.*, 34 (1991) 1925–1934.

Pavliv, L., Freebern, K., Wilke, T., Chiang, C.C., Shetty, B. and Tyle, P., Topical formulation development of a novel thymidylate synthase inhibitor for the treatment of psoriasis. *Int. J. Pharm.*, (1994) in press.

Snyder, L.R., Glajch, J.L. and Kirkland, J.J., *Practical HPLC Method Development,* Wiley, New York, 1988, pp. 71–83.

Snyder, L.R. and Kirkland, J.J., *Introduction To Modern Liquid Chromatography,* 2nd Edn, Wiley, New York, 1979, pp. 16–82.

The National Formulary XVII, USPC, 1990, Suppl. 6, pp. 2957–2958.

US Pharmacopeia XXII, USPC, 1990, ⟨621⟩ Chromatography, pp. 1558–1568, ⟨1225⟩ Validation of Compendial Methods, pp. 1710–1712.