Automated Sample Preparation in Quality Control of Eye-Drop Formulation

Application Note

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Abstract

The Agilent 7696A Sample Prep WorkBench with WeighStation was used in a quality control method for eye-drop formulations. Automated sample preparation included the preparation of calibration standards (four levels) containing two active ingredients, and the preparation of eye-drop formulation samples by weighing and dilution. The two sequences were fully automated. The analyses show excellent repeatability and linearity.

Introduction

The Agilent 7696A Sample Prep WorkBench with WeighStation was used for the determination of two active ingredients, dexamethasone and chloramphenicol, in eye-drop formulations. Dexamethasone is one of the most potent corticosteroids; it is 5 to 14 times more potent than prednisolone and 25 to 75 times more potent than cortisone and hydrocortisone. The addition of chloramphenicol, a broad-spectrum antibiotic, to dexamethasone leads to a combination that yields excellent results in inflammation of the anterior uvea (iritis, iridocyclitis). Eye-drop formulations against inflammation can contain one or both ingredients and are typically offered as a viscous aqueous solution.

The typical analytical QC procedure consists of 1) preparation of a 4-level calibration series containing the active ingredients in water (aqueous mobile phase) and 2) dilution of an amount of the eye-drop formulation in water (aqueous mobile phase). As illustrated in Table 1A, sample preparation is normally done in volumetric flasks, starting from the preparation of two stock solutions, containing respectively dexamethasone and chloramphenicol. The resulting calibration solutions contain both ingredients in a concentration range between 16 and 64 µg/mL dexamethasone and between 80 and 320 µg/mL chloramphenicol. Samples are typically 25-fold diluted in aqueous mobile phase. After sample preparation, calibration standards and samples are analyzed by HPLC with UV detection. Since the standard solutions are not stable, they are typically prepared each time a series of samples has to be analyzed.

In this application note, the automation of the preparation of the calibration standards and the dilution of the samples is described. The general method layout is shown in Table 1B. WeighStation was used to track the exact calibration standard concentrations and to measure sample amounts.

Experimental

Chemicals

Dexamethasone and chloroamphenicol were obtained from Sigma-Aldrich (Beerse, Belgium). Water and acetonitrile (AcCN) were HPLC grade (BioSolve, The Netherlands). Phosphoric acid and sodium hydroxide were from Sigma-Aldrich.

Configuration

The Agilent 7696A Sample Prep WorkBench was equipped with two Agilent 7693A Automated Liquid Samplers. The front injector contained an enhanced syringe carriage with a 500-µL syringe (p/n G4513-60561). The back injector contained an enhanced syringe carriage with a 50-µL syringe (p/n 5183-0314).

WorkBench method

Resource layout

Figure 1 shows the resource layout for the WorkBench. Mobile phase A (0.3% phosphoric acid in water, adjusted to pH = 3 with sodium hydroxide) and acetonitrile are placed as 'Chemical resource' in positions 81-150 and 71-72 respectively. Solvent in vials A1 and B1 for front and back tower was water. All vials in which the standard dilutions are prepared are defined as Empty Container.

WorkBench method program steps

The 7696A Sample Prep WorkBench program for the preparation of the four calibration standards is shown in Figure 2. After weighing the empty vials, the system is placed on pause to allow the addition of solid standards. After this, the automatic process is resumed and solvent is added to give calibration standards at four levels. The lowest level is prepared in six-fold.

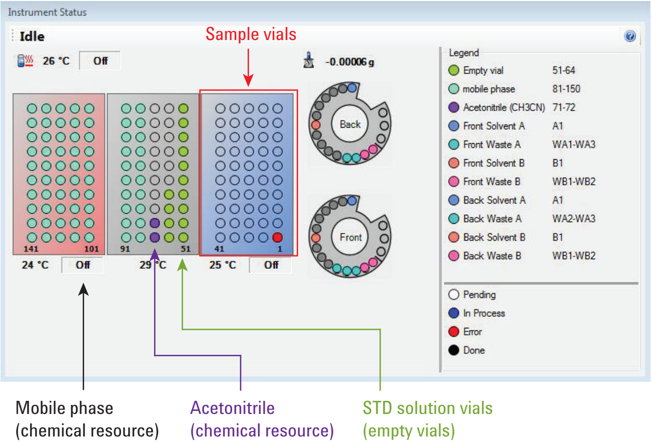
Table 1A. Classical Sample Preparation (USP or EP Methods)

	Code	Preparation stock solutions	Target conc (µg/mL)
Calibration standards	DEX Stck	weigh 10 mg dexamethasone in a 25-mL vial + add 25 mL water	400
	CLO Stck	weigh 10 mg chloramphenicol in a 25-mL vial + add 25 mL water	400
		Preparation standard solutions	**Target conc (µg/mL) dexamethasone/chloramphenicol**
	LVL1	1 mL Dex Stck + 5 mL CLO Stck in 25 mL mobile phase	16/80
	LVL2	2 mL Dex Stck + 10 mL CLO Stck in 25 mL mobile phase	32/160
	LVL3	3 mL Dex Stck + 15 mL CLO Stck in 25 mL mobile phase	48/240
	LVL4	4 mL Dex Stck + 20 mL CLO Stck in 25 mL mobile phase	64/320
		Preparation eye-drop formulation samples	
Samples	SAM	1 mL or 1 g eye-drop sample + 25 mL mobile phase	

Table 1B. Agilent 7696A Sample Prep WorkBench Sample Preparation

	Code	Preparation stock solutions	Target conc (µg/mL)
Calibration standards	DEX Stck-1	weigh 4 mg dexamethasone in a 2-mL vial * + add 1 mL AcCN	4,000
	DEX Stck	150 µL DEX Stck-1 + 1,350 µL mobile phase (1/10 dilution)	400
	CLO Stck-1	weigh 4 mg chloramphenicol in a 2-mL vial * + add 1 mL AcCN	4,000
	CLO Stck	150 µL CLO Stck-1 + 1,350 µL mobile phase (1/10 dilution)	400
		Preparation standard solutions	**Target conc (µg/mL) dexamethasone/chloramphenicol**
	LVL1	20 µL Dex Stck + 100 µL CLO Stck + 380 µL mobile phase	16/80
	LVL2	40 µL Dex Stck + 200 µL CLO Stck + 260 µL mobile phase	32/160
	LVL3	60 µL Dex Stck + 300 µL CLO Stck + 140 µL mobile phase	48/240
	LVL4	80 µL Dex Stck + 400 µL CLO Stck + 20 µL mobile phase	64/320
		Preparation finished product samples	
Samples	SAM	1 eye-drop sample* + 1 mL mobile phase (approximately 35–40 mg)	

*solid powder or viscous liquid is added manually while Agilent 7696A Sample Prep WorkBench is on hold. This is followed by a weighing step.



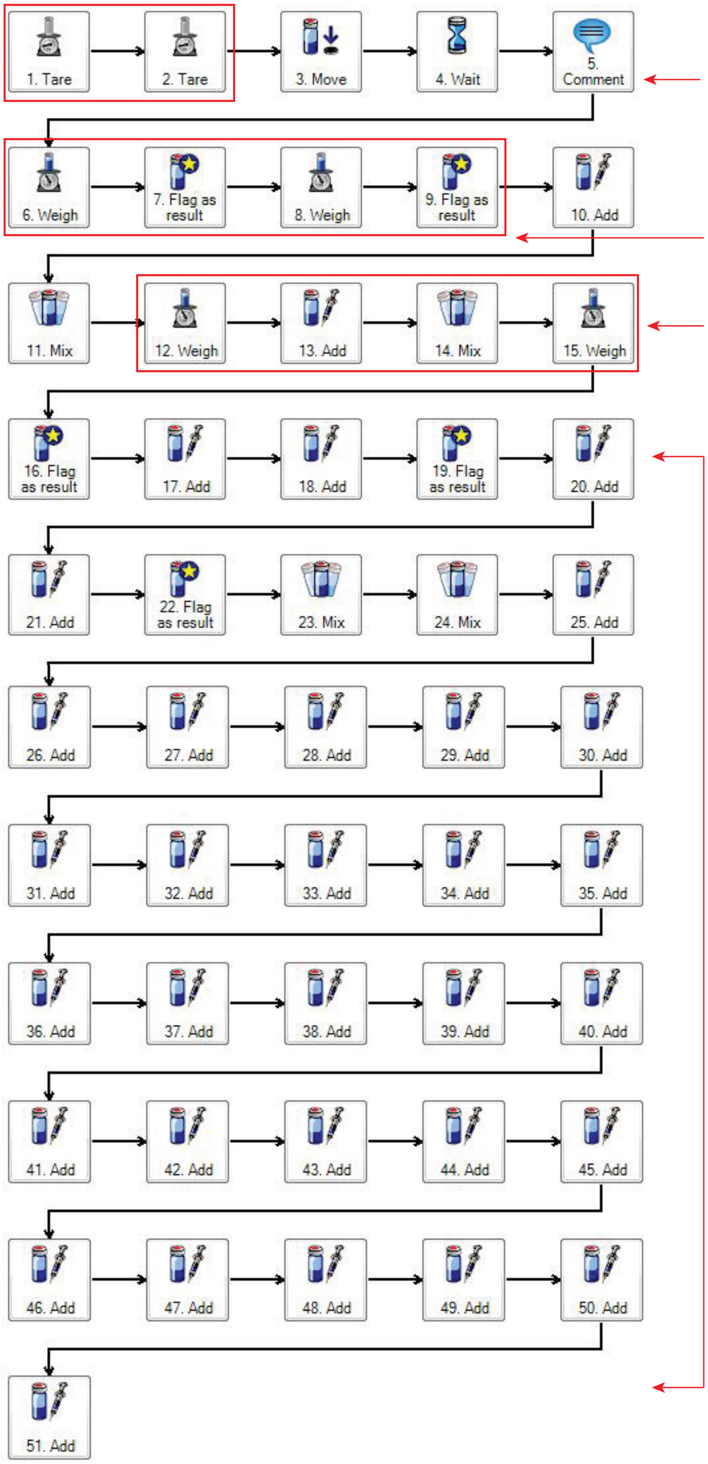
Legend

Empty vial	51-64
mobile phase	81-150
Acetonitrile (CH3CN)	71-72
Front Solvent A	A1
Front Waste A	WA1-WA3
Front Solvent B	B1
Front Waste B	WB1-WB2
Back Solvent A	A1
Back Waste A	WA2-WA3
Back Solvent B	B1
Back Waste B	WB1-WB2

○ Pending
● In Process
● Error
● Done

Figure 1. Agilent 7696A Sample Prep WorkBench resource layout for the serial dilution of dexamethasone and chloramphenicol.

Tare DEX stck-1 and CLO stck-1 vials



Press **PAUSE** on the Agilent 7696A Sample Prep WorkBench and add standard (approximately 40 mg). Press **START** to resume.

Weigh DEX stck-1 and CLO stck-1 vials (accurate standard weighing).

Weigh exact amount of added solvent.

Dilution

Figure 2. *Graphical flow chart used to program Agilent 7696A Sample Prep WorkBench method setup for calibration standards.*

An additional sequence is prepared for the dilution of the samples: empty vials are tarred and the system is put on hold. One drop of viscous sample is added to each vial. This requires a manual action since the viscosities of the samples do not allow transferring the liquids using syringes. The workbench is then resumed. The vials are again weighed (Figure 3) and the exact sample amounts are calculated and reported in the sequence report. Finally, solvent is added for sample dilution (see method lay-out in Figure 4).

Both programs for the preparation of the calibration standards and for the preparation of the samples can be programmed in one sequence.

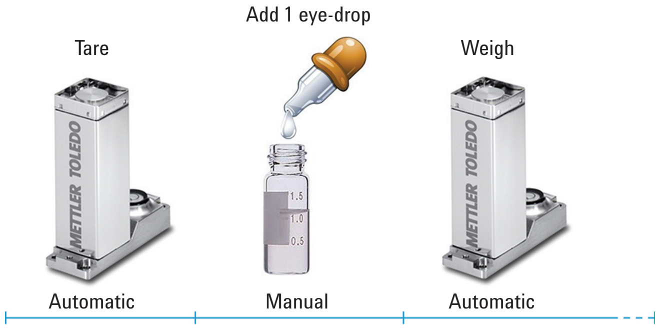
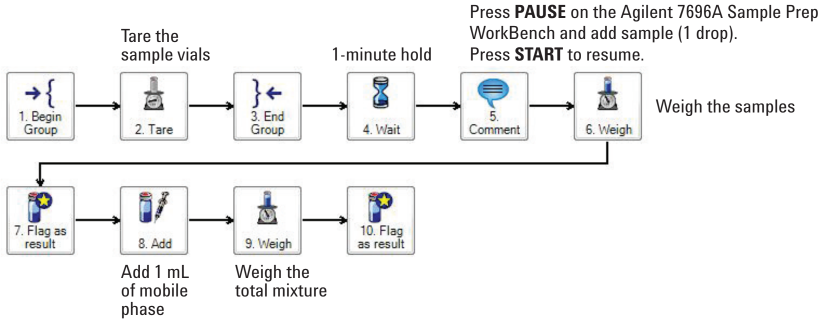


Figure 3. Illustration of taring, sample addition, and weighing of the sample amount.



1. Begin a group.
2. Tare the sample.
3. End the group.
4. Wait 1 minute 0 seconds.
5. Press **PAUSE** on the WorkBench. Press **START** after adding the sample.
6. Weight sample.
7. Flag the sample as RESULT.
8. Add 1,000 µL of the mobile phase to the sample at the front tower (washes, pumps).
9. Weigh the sample.
10. Flag the sample as RESULT.

Figure 4. Agilent 7696A Sample Prep WorkBench method setup for samples.

Experimental conditions for HPLC analysis

Analyses were performed on an Agilent 1290 Infinity HPLC System. Separation was performed on a Poroshell 120 column (2.1 mm × 400 mm, 2.4 µm dp (p/n 695775-902)). Mobile phase A was 0.3% phosphoric acid in water (adjusted to pH = 3 with sodium hydroxide) and mobile phase B was acetonitrile. A gradient from 20% B (0 minutes) to 50% B (5 minutes) was used. Flow rate was 0.5 mL/min. Injection volume was 1 µL and detection was done by UV at 254 nm.

Results and Discussion

The chromatograms corresponding to the four calibration levels are overlaid in Figure 5. The calibration curves are given in Figure 6. The linearities for both dexamethasone and chloramphenicol are excellent ($R^2 > 0.999$).

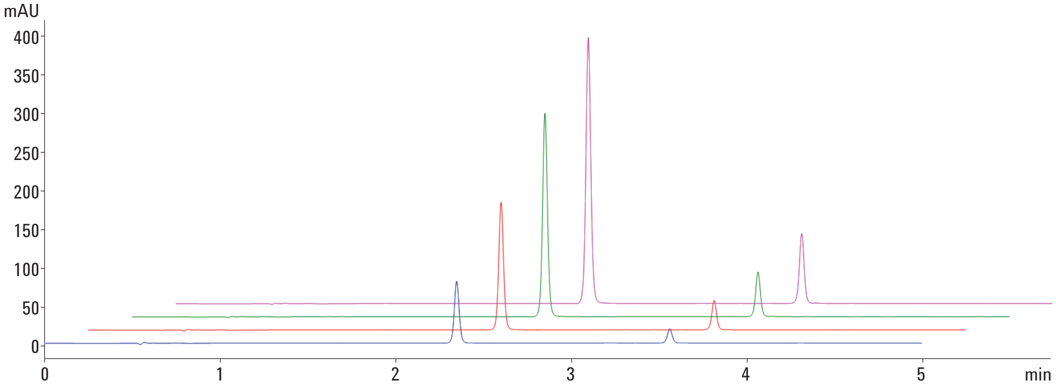


Figure 5. Chromatograms (overlay with X/Y-axis offset) of four calibration levels of chloramphenicol (2.35 minutes) and dexamethasone (3.55 minutes).

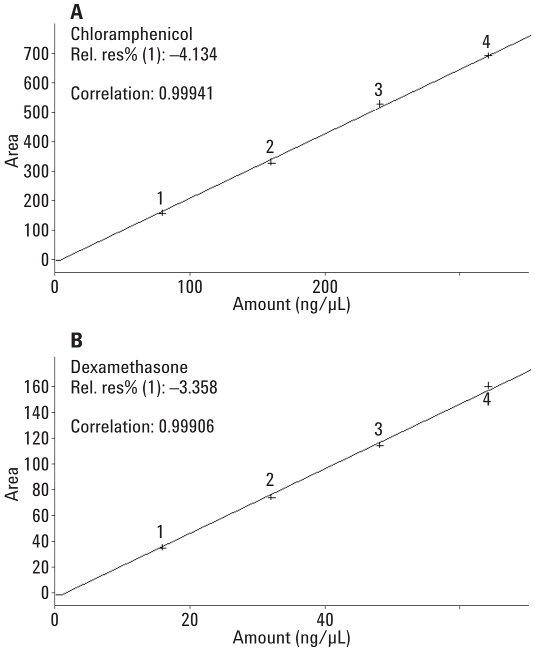


Figure 6. Calibration curves for chloramphenicol (2.35 minutes) and dexamethasone (3.55 minutes) obtained by automated calibration standard preparation using the Agilent 7696A Sample Prep WorkBench.

The repeatability of sample preparation was evaluated by preparing the lowest calibration sample in six-fold. The chromatograms are overlaid in Figure 7 and show excellent repeatability, with RSDs of 1.5% for chloramphenicol and 2.7% for dexamethasone, respectively.

Next, three types of samples were analyzed. The chromatograms are shown in Figure 8. Sample A contains dexamethasone, sample B contains chloramphenicol and sample C contains a combination of both solutes. For all three samples, correct amounts of the active ingredients were detected (values all between 90% and 105% of labelled concentration). The analyses of different sample types that were prepared in the same sequence also revealed that no cross-contamination was observed (no chloramphenicol in sample only containing dexamethasone and *vice versa*).

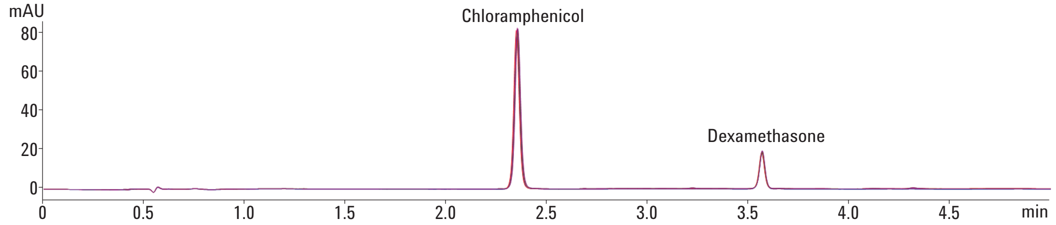


Figure 7. Overlay of 6 chromatograms of six individually prepared calibration solutions of chloramphenicol (2.35 minutes) and dexamethasone (3.55 minutes) obtained by automated calibration standard preparation using the Agilent 7696A Sample Prep WorkBench. The percent relative standard deviation for chloramphenicol and dexamethasone are 1.5% and 2.7%, respectively.

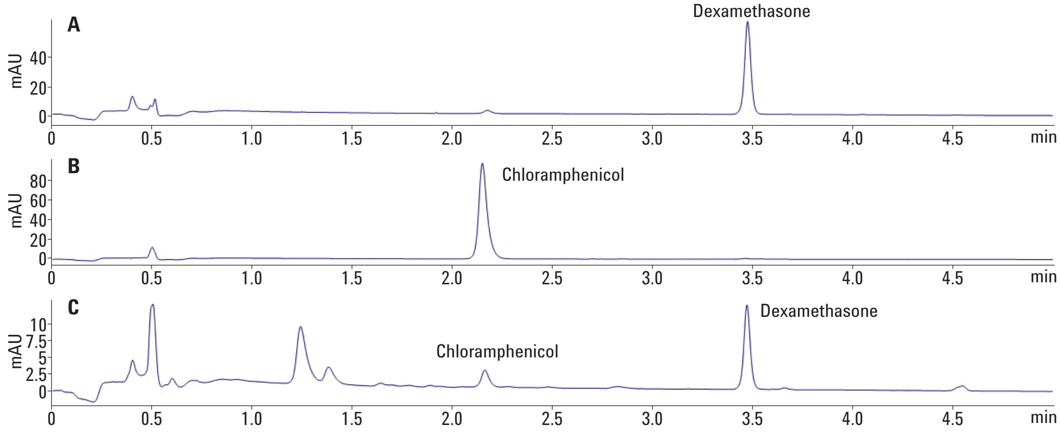


Figure 8. Chromatograms obtained for three eye-drop formulation types.

The repeatability of sample preparation is demonstrated in Table 2, showing the measured concentrations, the sample weights and the final concentrations of chloramphenicol in sample type B. This sample was prepared in 6-fold. The relative standard deviation of the measured amount is lower than 1%.

In addition to these quantitative data, note that the total solvent consumption is drastically reduced, no volumetric glassware is used, and hardly any solutions are wasted.

Table 2. Repeatability of Sample Preparation for Eye-drop Formulation Type B

	Measured conc (µg/mL)	**Sample weight (mg)**	**Corrected conc (µg/mg)**
SAM-rep01	124	27.72	4.59
SAM-rep02	121	26.95	4.61
SAM-rep03	114	25.41	4.61
SAM-rep04	103	23.07	4.57
SAM-rep05	143	32.58	4.53
SAM-rep06	138	31.27	4.53
		RSD (%)	**0.74**

Conclusions

The Agilent 7696A Sample Prep WorkBench with WeighStation was successfully applied in the automated preparation of calibration samples and sample dilutions. The viscous nature of the samples did not allow volumetric dilution with syringes, but using the weighing station, exact sample masses were determined. After dilution, accurate determination of the active ingredients was possible.

For More Information

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