Journal of Pharmaceutical & Biomedical Analysis
Vol. 8, Nos 8–12, pp. 995–998, 1990
Printed in Great Britain

0731–7085/90 $3.00 + 0.00
Pergamon Press plc

Short Communication

The determination of small quantities of water in single vials of pharmaceutical products by flow injection analysis*‡

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Keywords: *Flow injection analysis; water determinations; Karl–Fischer reagent; freeze-dried injections.*

Introduction

Many moisture sensitive drugs are freeze-dried in unit dose vials and reconstituted with a solvent just before administration. The amount of residual water in the freeze-dried product is controlled in order to ensure physical and chemical stability. The lyophilized solid tends to be hygroscopic and it is preferable to determine the residual water *in situ* in the vial rather than risking exposure to atmospheric moisture during the transfer operation.

Flow injection analysis (FIA) has been applied to determine small quantities of water in individual vials of freeze-dried pharmaceuticals. A dilute solution of Karl–Fischer reagent is pumped through a simple FIA manifold and the absorbance monitored spectrophotometrically at 615 nm. Single vials of the product are reconstituted with a suitable anhydrous solvent and injected into the flowing stream. Water in the injected solution reacts with the Karl–Fischer reagent and generates a negative peak. As the peak heights are directly proportional to the concentration of water in solution, a comparison of heights of sample and standard solutions, after subtraction of a blank due to the trace water content of the solvent, enables quantitation of the amount of water in the vial.

The method has been customized for the determination of water in reconstituted solutions up to 0.21% v/v but this range can be extended by increasing the concentration of Karl–Fischer reagent. An application to a typical product, Acyclovir IV for infusion, is described.

This method is quicker than micro Karl–Fischer titration (100 compared to typically 30 determinations per hour) and needs only a small amount of the reconstituted solution. Consequently the solution can be analysed for the active ingredient and degradation products in parallel with the water determination.

Experimental

Apparatus

The Flow Injection manifold is shown schematically in Fig. 1. The development apparatus consisted of a Gilson Minipuls 2 Peristaltic pump equipped with silicon rubber tubing, a Magnus Scientific M7100 autosampler, a Cecil Instruments CE 272 spectrophotometric detector equipped with a 10 mm flow cell and a Bryans Southern Instrument 28000 chart recorder. Teflon tubing (0.5 mm i.d.) was used for all connections and as reaction tubing.

*Presented at the "Second International Symposium on Pharmaceutical and Biomedical Analysis", April 1990, York, UK.

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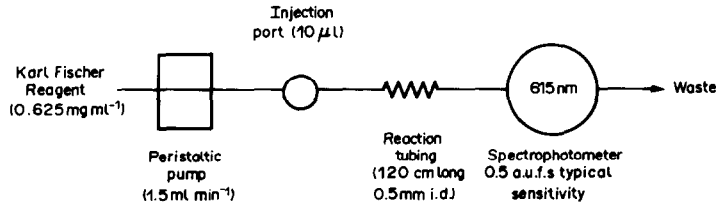

Figure 1
Schematic flow injection manifold.

FIA conditions.

Reagent:	0.625 mg ml^{-1} Karl–Fischer reagent
Flow rate:	Approx. 1.5 ml min^{-1}
Injection volume:	10 μl
Reaction tubing:	120 cm long PTFE tubing (0.5 mm i.d.)
Wavelength of detection:	615 nm
Sensitivity:	0.5 a.u.f.s.

The technique of FIA has previously been applied to the determination of water in organic solvents [1–4]. This procedure has been extended to determine the small quantities of water in individual vials of freeze-dried pharmaceuticals.

Determination of water in Acyclovir IV for infusion

Reagents. Methanol was specially dried for Karl–Fischer titration and ethanediol was Analytical reagent grade (both available from BDH Chemicals). Ethanediol was dried by standing over molecular sieve type 3 Å (ex BDH) pre-heated at 250°C. The reconstitution solvent was a 20% v/v solution of ethanediol in methanol.

Karl–Fisher reagent Hydranal-composite 5 (5 mg H_2O per ml, from Riedel-de Haën) was diluted with specially dried methanol to give a reagent strength of 0.625 mg ml^{-1}. This reagent strength is suitable for the examination of reconstituted vial contents containing up to 0.28% v/v water.

Sample solution. Reconstitute the contents of an Acyclovir 250 mg IV vial with 10.0 ml of anhydrous solvent using a precision syringe and needle inserted into the rubber plug. Transfer the contents into autosampler vials and seal.

Standard solution. Prepare a 0.14% v/v water in solvent solution as the standard. This is equivalent to the maximum water concentration level permitted in a reconstituted Acyclovir 250 mg IV vial.

Blank. The blank consists of the solvent used to prepare the sample and standard solutions.

Karl–Fisher linearity standard. It is desirable to ensure that the Karl–Fischer reagent has not degraded from occasion to occasion due to uptake of atmospheric moisture. For this purpose prepare a standard at a concentration 150% of the upper limit expected from the sample solutions (0.21% v/v). Inject this standard and confirm the linearity of the reagent at the start of a new analysis.

Procedure. Load all solutions into sealed autosampler vials taking appropriate precautions to prevent the uptake of atmospheric moisture. Pump the Karl–Fischer reagent through the manifold until a stable baseline is obtained.

Inject portions of the standard solution, the blank and the Karl–Fischer linearity standard, until the reproducibility of the measured peak heights are satisfactory. Confirm the linearity of the reagent by comparison of the linearity standard with the standard and blank.

Sequentially inject the required number of sample solutions concluding the analysis with bracketing injections of blank and standard solutions. Measure the peak heights, correct the sample and standard results for the blank response and then, by comparison, calculate the water contents of the individual vials.

Results and Discussion

Method development

Work concentrated on determination of small amounts of water in a typical freeze-dried

product, a formulation containing 250 mg of Acyclovir per vial, manufactured by The Wellcome Foundation Ltd. The level of water in this product is limited to less than 14 mg per vial (equivalent to a 0.14% v/v solution when reconstituted in 10 ml solvent) and hence the method, particularly the injection volume and strength of Karl–Fischer reagent, has been tailored towards this. The method was designed to be linear at concentrations up to 0.28% v/v. Linearity was normally confirmed up to 0.21% v/v, 150% of the limit. Higher water concentrations can be determined by using a more concentrated Karl–Fischer reagent. Typical FIA traces for standard, sample and blank solutions are shown in Fig. 2.

Peak height was studied as a function of the reaction coil length at the optimal flow rate of 1.5 ml min^{-1}. Results with the confidence intervals ($P = 0.05$) plotted as error bars (12 injections) at each coil length are shown in Fig. 3.

A coil length of 120 cm gave an optimal response. It is considered that at shorter coil lengths either the dispersion or the reaction time is insufficient for complete reaction to occur, hence the peak heights are smaller. At coil lengths above the optimum of 120 cm, dispersion of the peak is the predominant factor and smaller peak heights again result.

At the wavelength of 615 nm the blank does not contribute to the absorbance and all measurements are within the normal range of the spectrophotometer.

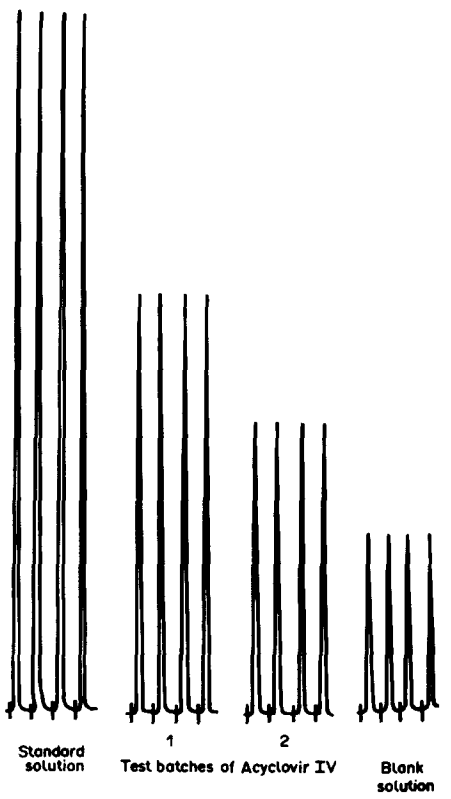

Figure 2
Typical FIA traces.

Validation

Validation has been carried out on the application of the method to Acyclovir IV for infusion, using a reconstitution solvent of 20% v/v ethanediol in methanol.

Linearity of the method. The linearity of the method over the water concentration range 0–0.28% v/v was confirmed by injecting a series of standard solutions. A linear response of peak height (mm) vs concentration (% v/v) was obtained with a slope of 716.8, a y intercept at −0.32, a correlation coefficient of 0.9999 and a standard error of the slope of 0.9227.

Recovery from a reconstituted sample solution. Known amounts of water were added to a reconstituted solution of Acyclovir to produce solutions equivalent to 0.07, 0.112, 0.14 and 0.168% water in solvent (50, 80, 100 and 120% v/v of the limit applied to this product). Results of 102.2, 102.3, 100.9 and 99.6% of the added water were recovered from each solution, respectively. The recovery from the product is satisfactory.

Precision. The repeatability of the method was assessed by carrying out 15 injections of a standard solution on a single occasion. A relative standard deviation of ±0.95% was obtained.

For reproducibility, two operators each examined four separate vials of the same batch of product. The mean, standard deviation and relative standard deviation obtained for the two operators (incorporating vial to vial variation) were 6.65, 0.181 and 2.72, respectively. The mean results obtained by both operators agreed well (6.63 and 6.67).

Application to batches. The method was applied to seven batches of product examined by FIA and the standard micro Karl–Fischer titration. The comparative results are given in Table 1.

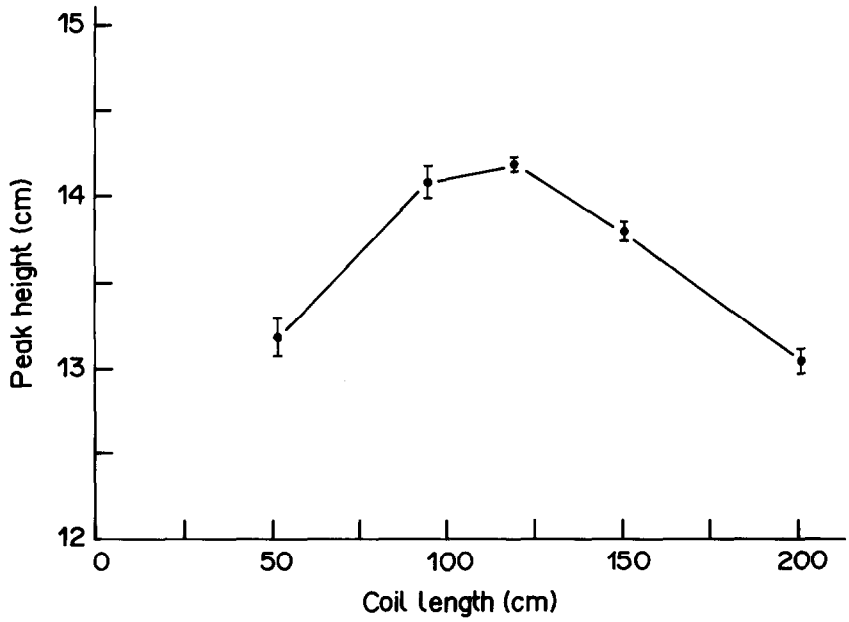

Figure 3
Graph showing peak height as a function of reaction coil length.

Table 1
Comparison of results (mg water per vial) obtained by FIA and micro Karl–Fischer titration

Batch	FIA Range	FIA Mean	Micro Karl–Fischer titration Range	Micro Karl–Fischer titration Mean
1	1.12–2.46	1.71	0.78–2.77	1.71
2	0.84–5.24	2.94	2.16–4.70	3.02
3	6.52–9.16	7.56	6.19–8.92	7.53
4	6.00–9.35	7.95	5.46–9.52	7.53
5	2.18–7.11	3.25	2.30–3.50	2.97
6	3.30–3.50	2.83	1.68–3.70	2.94

These results show the equivalence of the FIA method compared to the currently employed micro Karl–Fischer titration procedure.

Conclusions

FIA has been applied to determine small quantities of water in individual vials of a freeze-dried pharmaceutical product. The method should be applicable to a wide range of freeze-dried products with minor modifications to the reconstitution solvent and Karl–Fischer reagent strength.

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[*Received for review 5 April 1990; revised manuscript received 19 April 1990*]