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The development and evaluation of a diluter–ultraviolet spectrophotometric analysis system for the determination of drugs in solutions and suspensions

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Abstract

An automated system has been developed and validated for the analysis of liquid formulations (solution and suspension). The system comprises a multi-place magnetic stirrer, a diluter and a diode array UV spectrophotometer. The system can handle a wide range of drug formulation concentrations (>200 mg ml^{-1}) and gives excellent precision and accuracy with no detectable carryover. The analysis acceptance parameters are user-definable and the analysis process, including interpetation and reporting of the data, is fully automated.

Keywords: Automation; Formulation analysis; Suspensions; UV spectroscopy

1. Introduction

Many toxicology studies involve the administration of a test compound in a liquid formulation which often takes the form of an aqueous suspension. The suspending agent is typically a water-soluble cellulose derivative (e.g. hydroxypropyl methyl cellulose or carboxymethyl cellulose) as a thickening agent with or without the addition of a wetting agent such as the detergent Tween-80. These formulations can be difficult to prepare due to the physical properties of the test material and, furthermore, continual stirring of the formulation is often necessary to maintain homogeneity. To confirm the accurate preparation of the formulation and its homogeneity, some form of chemical analysis is usually carried out. This can involve a chromatographic procedure or more commonly simple UV analysis. Given the high concentrations of the test compound, often up to 400 mg ml^{-1} (40% w/v), coupled with the high sensitivity and narrow dynamic range of most instrumental methods, considerable dilution of the samples is required prior to analysis proper.

Although the dilution steps can be partially automated, much analysis is still carried out manually using volumetric glassware. Because of the difficulty in accurately pipetting viscous suspensions, initial dilutions are often carried out gravi-

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metrically. These procedures are not only tedious and time-consuming, but involve the use of large amounts of solvents and the attendant risks in using glass pipettes, etc. Once the samples have been prepared they still have to be analysed manually and the results collated, interpreted and reported.

The instrument described here allows total automation of the dilution, analysis, interpretation and reporting procedures. The instrument is highly versatile and the acceptance and rejection parameters are fully user-definable.

2. Experimental

2.1. Major equipment

The system consists of a Hamilton Microlab 2200 diluter, a Hewlett Packard 8452 diode array UV spectrophotometer, a 60-plate magnetic stirrer (Camlab Ltd., Cambridge, UK) and a standard 486 personal computer (PC). The Hamilton diluter gives an accuracy of better than $\pm 1.0\%$ and a precision of better than 0.3% when dispensing volumes of 0.500 ml using a 1 ml syringe. This level of performance was considered to be more than satisfactory for the anticipated range of sample types and concentrations. The incorporation of a multi-place magnetic stirrer ensured that all samples remained homogeneous during sampling and that the resultant dilutions were adequately mixed prior to UV analysis.

The HP 8452 diode array spectrophotometer was selected for spectral measurements since it offered ready access to the sample compartment, gave rapid spectral measurements and was simply controlled via a IEEE 488 instrument interface.

Of the 60 places on the magnetic stirrer, one place is reserved for a drug standard solution and 19 are used for formulation samples (including control formulations). The additional 40 places are reserved for vials into which the standard and samples are diluted (up to two dilution vials per sample depending on concentration).

The whole system is controlled through a single PC which also accepts the data from the user and performs the necessary calculations and data assessment.

2.2. Reagents and consumables

Methanol is used as the system diluent since it is a relatively good solvent, has low UV background and, in contrast to acetonitrile, it does not precipitate the cellulose-based materials from the formulation vehicles at low dilutions. However, in order to improve its solubilising power and give reproducible UV spectra, in a number of assays it was found to be necessary to add a small amount of acid or base to the methanol diluent. All standards, samples and controls are presented to the system in 20 ml glass scintillation vials. A minimum of 4 ml of sample is required for analysis.

2.3. Software

A schematic diagram showing the various software modules and how they interface with the hardware is given in Fig. 1. The main program has several sub-modules, including the Hamilton Eclipse program which controls the diluter. Referencing and scanning were carried out using a series of macros which interfaced with the Hewlett Packard command library. The programm module Spectrum which was written by Hamilton carries out the spectral match using the method of Kohn [1] and provides the spectral display.

For every compound requiring analysis a compound data file is first created within System Manager and this is stored on disc. This file contains the $A^{1\%}_{1\,cm}$ value for the compound, the

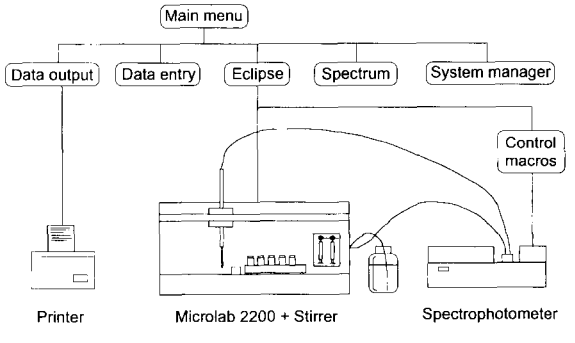

Fig. 1. Schematic diagram showing the major hardware components and software modules of the diluter–UV analysis system.

spectral range, the measurement wavelength, the spectral match factor and the concentration which gives an absorbance of 0.6 AU.

2.4. System operation

In operation the user identifies the compound to be assayed and enters the necessary details, e.g. the study number, the concentration of the standard, the number of dose groups, the number of samples per group, and the identifiers for the samples and controls. The system then calculates the necessary dilutions for the standard and samples to be analysed. The dilution factor is that required to ensure that the diluted standard and all the diluted samples give an absorbance of 0.6 AU at the pre-selected measurement wavelength. This value was selected since the reproducibility of the UV spectrophotometer is ± 0.005, thus ensuring that the absorbance measurement does not add significantly to the overall imprecision. The system then provides an Analysis Schedule which indicates how the samples are to be arranged on the stirrer and the number of dilution tubes required for each sample. Depending on the concentration and the $A^{1\%}_{1\,cm}$ value some standards and samples require a second dilution to bring the absorbance down to 0.6 AU. Each sample, standard or control is placed in a sample rack which sits over the 60-place magnetic stirrer. The homogeneity of the suspensions is maintained by the use of a small magnetic flea (≈ 12 mm in length).

The first stage in the analytical process proper involves a system check through analysis of a standard solution. The standard is diluted and the diluted solution aspirated and transported into the UV cell where the spectrum is determined and the absorbance at the predefined wavelength measured. The actual concentration of the standard is calculated using the pre-programmed $A^{1\%}_{1\,cm}$ value and compared with the prepared concentration. These two values must be within a predefined percentage of one another (typically 5%) for analysis to continue. If this system check fails the operator is given the options of re-sampling or aborting the run.

For every batch of samples at a given concentration, a sample of control vehicle must be included. This is necessary since the vehicle has significant UV absorbance around 234 nm. The control vehicle is diluted in the same manner as the samples and the spectrum recorded and stored for use as a reference or background spectrum. Each sample within the batch is then diluted in turn. Depending on the dilution ratio and the nature of the material being diluted there are two delay periods prior to the next stage. Mixing of an aqueous-based vehicle with methanol (in similar proportions) is an exothermic process which also results in outgassing. Therefore, to allow attainment of thermal equilibration after dilution there is a mixing time and a de-aeration time after the mixing is ceased. Both these times are related to the dilution factor: the lower the dilution factor, the longer the mixing and de-aeration times. After the appropriate delay the diluted sample is aspirated. The system actually takes four small aliquots (50–300 μl) separated by a small air bubble (50 μl) and these are transported into the spectrophotometer. The last aliquot is scanned against the reference spectrum. The absorbance at the pre-defined wavelength is again measured and the concentration in the original sample calculated. The sample spectrum is also compared statistically against that of the standard and a match factor computed.

At the end of the analysis the system is flushed and a spectral reading taken from the flow cell to test for contamination. The results are then printed. These include the sample number, its identity along with the determined concentration, and a *Pass* or *Fail* flag. This acceptance or rejection of the result is based on the sample concentration being within pre-defined acceptance limits (typically $\pm 10\%$ of the stated concentration). The spectral match factor is also printed along with a *Pass* or *Fail* flag based on the match factor being above a predefined value, typically 995.

3. Results and discussion

3.1. System capability

Through the use of an integral 60-place magnetic stirrer all suspension formulations can be stirred continuously during analysis, with the stirrer being switched off momentarily during the

actual sampling of the formulation.

The handling capability of the system, with respect to the concentration of the sample, is very dependent on the UV properties of the compound. Using a 1 ml syringe for sample handling and a maximum sample size of 22 ml, the diluter is capable of making single-stage dilutions up to 140-fold with an accuracy of better than 4%. When a two-stage dilution is carried out the overall dilution factor can be as high as 50 000. Thus with a typical compound having an $A^{1\%}_{1\,cm}$ value of 500, concentrations in excess of 500 mg ml^{-1} can be reliably assayed. However, the solubility of the compound in question must be such that it is soluble in the diluent having undergone a single-stage dilution of 1/140.

There are some limitations at low concentrations (typically <1 mg ml^{-1}) when the compound has a very low $A^{1\%}_{1\,cm}$ value. Where these factors combine so as to require a low dilution, i.e. <10, then erroneous results can be obtained. This appears to be due to Tween-80 (a detergent) from the formulation vehicle reducing the surface tension of the diluted sample which causes dispersion of the sample in the liquid transfer lines during transport to the spectrophotometer. The resulting break-up of the liquid "slug" results in the introduction of air bubbles into the cell during the measurement stage. This occurs despite the sample slug being sandwiched between two air bubbles during the transport process.

Careful optimisation of the liquid handling parameters allowed accurate dilutions as low as 1/12 to be carried out even with Tween-80 in the formulations. This was attained by controlling the speed of transfer in the liquid lines, the volume of liquid in each slug, and the size of the air gaps between slugs and by minimising the length of the liquid transfer lines. The best approach was found to involve four aliquots of the diluted sample (approximately 300 μl each) separated by an air gap of approximately 50 μl. The sample slug corresponding to the last of the four aliquots was always intact and hence this was scanned.

The current limitation with regard to sample numbers (20 in total including standard and controls) is dictated by the capacity of the 60-place stirrer. Only around one-third of the base area of the Hamilton 2200 is used however and if necessary the capacity could be increased several fold.

3.2. System validation

3.2.1. Accuracy

The accuracy of the automated procedure was determined by comparing the results for 27 separate analyses involving six different compounds, with the concentrations varying over the range 0.1–200 mg ml^{-1}. The data showed a mean percentage difference between the manual and automated results of 0.83% (range 6.3%– −5.1%). The data were also analysed by linear regression (automated on manual) which gave a slope ($\pm$S.E.) of 0.9667 ± 0.0033, an intercept ($\pm$S.E.) of 0.324 ± 0.276 and a correlation coefficient of 0.9997. Statistical analysis of these data [2] showed the slope and intercept to be statistically indistinguishable (at the 95% confidence interval) from 1.00 and zero respectively. These results show the system to have an acceptable degree of accuracy with no serious bias.

3.2.2. Precision

The reproducibility of analysis has been found to be dependent on the characteristics of the sampling probe. As expected, much better precision was obtained when a narrow-gauge probe, internal diameter 0.559 mm (0.022 in), was used. Despite the small diameter, blocking of the probe with solid material was not found to be a problem. The overall assay precision was investigated for four Zeneca development compounds. Compound A had a relatively low $A^{1\%}_{1\,cm}$ value which, in combination with the low concentration analysed, necessitated small dilutions. Two of the compounds, C and D, were selected since they produced suspensions which were quite viscous, especially at high concentration. The high density of these compounds, together with their propensity to settle out, further added to the difficulty of handling.

Table 1
Performance data for the automated diluter–UV analysis system showing assay precision over a range of sample concentrations and dilutions

Compound	$A^{1\%}_{1cm}$	Conc. (mg ml^{-1})	System dilution factor	No. of samples	No. of batches	Intra-assay RSD (%)	Inter-assay RSD (%)	Total assay RSD (%)[a]
A	111	0.72	12	8	4	1.1	0.59	1.2
B	36.5	2.4	14	4	4	1.3	0.022	0.99
B	36.5	11.8	69	4	4	1.3	0.41	1.39
B	36.5	107	629	4	4	1.3	0.80	1.51
C	607	200	20000	8	4	2.8	1.5	3.1
D	1504	200	50000	8	4	1.2	1.2	3.3

[a] Includes intra- and inter-assay contributions.

The resulting data were analysed using one-way analysis of variance (ANOVA) and the results are presented in Table 1. It is clearly seen that the precision, both intra and total (i.e. including intra- and inter-assay contributions), is excellent with total assay RSDs of $< 3\%$.

3.2.3. Carryover

Carryover in the system is unlikely for a number of reasons. The samples are assayed in groups of the same concentration with the concentration increasing from one group to the next. However, the last sample from one group could adversely affect the control sample which is used to generate a reference for the next group. The possibility of this occurring was investigated by assaying several samples where the concentration varied between high (100 and 200 mg ml^{-1}) and zero. This was carried out with two compounds of differing physicochemical properties and $A^{1\%}_{1cm}$ values. The results of this work indicated that carryover was non-existent. The blank samples assayed after a high concentration sample typically gave concentrations which were between -0.5 and $+0.5\%$ of the result for the previous sample. These results show that carryover is minimal or non-existent and the variability that is observed corresponds to the reproductibility of the spectrophotometer.

As a further measure to guard against contamination, the whole system is thoroughly flushed with 10 ml of diluent at the end of an analysis and a spectral reading is taken at the measurement wavelength of the preceding analysis.

3.2.4. Special comparison

Spectral comparison is carried out using a similarity factor [1]. This is a measure of the goodness of fit between the standard and sample spectra. It is calculated using the least-square fit coefficients for absorbance pairs across a pre-defined spectral range. This method forms the basis of spectral matching in many commercial UV-diode array detection systems and is well tested and available to the present authors. A similarity factor of 1000 indicates identical spectra. Match values of around 995 or greater were typically obtained when comparing known samples and standards, indicating a very good match. Thus the system is also able to provide some confirmation of the identity of the samples as well as a measure of possible contamination.

3.3. Sample throughput and solvent usage

The length of time taken for a typical analysis (one standard, three controls and three samples) is around 30 min. Comparable manual analysis would take around 45 min, i.e. 50% more time. Furthermore, with manual analysis the operator is fully occupied for the whole analysis period whilst, with the automated method, once the samples have been loaded the operator is free to carry out other tasks.

The volume of solvent used with the automated system is relatively small, typically 175 ml for an average batch of samples. This represents a very significant saving over the 700–800 ml which would be used for comparable manual analysis.

4. Conclusions

The described system allows the full analysis of liquid formulations including suspensions which require continuous stirring. The system has a very wide dynamic range in terms of the dilutions it can accurately perform (1/10 to 1/50 000), giving very great flexibility in terms of the range of formulation concentrations that can be handled. The system shows good accuracy and precision with the minimum of carryover beween samples. The whole analytical process is automated including interpretation and reporting. Not only does the system provide quantitative concentration data but, through the use of spectral matching, a measure of compound identity or gross contamination is also obtained. Furthermore, there is a significant saving in solvent consumption (at least fourfold) with an increase in throughput and a reduction in the number of hours spent on analysis compared with manual procedures.

References

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