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High pass digital filtering applied to quantitative UV spectrometry

RON JONES* and BARRY S. LEADLEY

Analytical Development Laboratories, The Wellcome Foundation Limited, Temple Hill, Dartford, Kent DA1 5AH, UK

Abstract: High pass digital filtering is a novel method of data treatment for quantitative UV spectrometry in the presence of broad interfering bands. The test and standard spectra are digitally filtered using the binomial filter to remove low frequency components and a least squares fit of the standard to the test is then carried out. This paper reports a preliminary evaluation of the method, including a theoretical comparison with derivative spectrometry and the successful application to a typical problem in pharmaceutical analysis, the assay of the decongestant pseudoephedrine hydrochloride in the presence of the antihistamine triprolidine hydrochloride.

The procedure should also be suitable for enhancing the specificity of other spectroscopic techniques.

Keywords: *UV spectrometry; high pass digital filtering; derivative spectrometry; pseudoephedrine; triprolidine.*

Introduction

UV spectrometry is a rapid and precise technique for quantitative analysis. However, it is of limited use for analysing mixtures because the bands are usually broad and overlapping. Various techniques have been introduced to increase the specificity, the most popular being derivative spectrometry. The conditions under which it may be used have been studied theoretically [1] and it has been applied extensively to pharmaceutical analysis where it minimizes interference either from wide overlapping bands or from excipients which scatter the incident light [2]. An advantage of the derivative method is that the spectra of the interferences do not enter into the calculation.

This paper presents an alternative method of data treatment, high pass digital filtering. It selectively removes the low frequency components of the spectrum and is suitable for the quantitative analysis of mixtures with broad interfering peaks. The spectra of the interferences do not enter into the calculation and need not be known. High pass filtering allows precise tuning of the filter width in order to retain lower frequencies of the spectrum, with a consequent increase in signal to noise ratio over derivative spectrometry.

*To whom correspondence should be addressed.

This paper presents the evaluation of high pass digital filtering for a typical problem in pharmaceutical analysis, the assay of the decongestant pseudoephedrine hydrochloride in the presence of the antihistamine triprolidine hydrochloride. The results are compared to a reference method, analogue second derivative spectrometry.

Theory

Theory of high pass digital filtering

The three-point binomial filter is used to smooth (low pass filter) the UV spectrum which is digitized with a fixed wavelength step. This involves either single or repeated convolution with the sequence (1,2,1)/4 and can be carried out either by retaining fixed end points or by losing the two extreme points on each pass of the filter. Subtracting the smoothed spectrum from the original gives a high pass filtered spectrum which has a reduced amplitude and, in the example given, has both positive and negative lobes. The bandwidth of the digital filter depends on the number of smooths applied to the subtracted spectrum. Increasing the number of smooths increases the bandwidth of the filter, retaining lower frequency components of the spectrum.

In common with derivative spectrometry, high pass filtering always causes a decrease in the signal to noise ratio which depends on the degree of smoothing. This is overcome by filtering the standard and test spectra identically, and then carrying out a least squares fit of the standard to the test spectrum. The theory of the least squares fit takes a particularly simple form for a single standard spectrum as follows:

Let $\boldsymbol{q}$ be an $n \times 1$ vector of absorbances q_i for the test solution measured at n equally spaced wavelengths.

Let $\boldsymbol{p}$ be the corresponding $n \times 1$ vector for the standard solution.

The least squares fit of $\boldsymbol{p}$ to $\boldsymbol{q}$ is given by the equation $\boldsymbol{q} = f\boldsymbol{p}$ where f is a scalar.

The residual vector $\boldsymbol{r} = f\boldsymbol{p} - \boldsymbol{q}$.

The sum of the squared residuals $R = \boldsymbol{r}^T\boldsymbol{r} = (f\boldsymbol{p} - \boldsymbol{q})^T(f\boldsymbol{p} - \boldsymbol{q}) = f^2\boldsymbol{p}^T\boldsymbol{p} - f\boldsymbol{q}^T\boldsymbol{p} - f\boldsymbol{p}^T\boldsymbol{q} + \boldsymbol{q}^T\boldsymbol{q}$ where T denotes a transposed vector.

To minimize R, $dR/df = O$

$dR/df = 2f\boldsymbol{p}^T\boldsymbol{p} - \mathbf{q}^T\boldsymbol{p} - \boldsymbol{p}^T\boldsymbol{q} = 0$

$\boldsymbol{q}^T\boldsymbol{p} = \boldsymbol{p}^T\boldsymbol{q}$ since both are scalars and rearranging gives $f = \boldsymbol{p}^T\boldsymbol{q}/\boldsymbol{p}^T\boldsymbol{p}$.

For calculation purposes it is more conveniently written as

$$f = \frac{\sum_{i=1}^{n} p_i q_i}{\sum_{i=1}^{n} p_i^2}.$$

Comparison with derivative spectrometry

Derivative assays are usually carried out by comparing deflections in the test and standard spectra. In this work high pass filtering was quantified by the least squares method. The difference is not significant, in that the least squares method can also be used with derivative spectra. The significant difference between the methods is in the treatment of the spectrum to remove interferences. A spectrum may be considered as a sum of sine and cosine waves of various frequencies, as produced by carrying out a

Fourier transform. A digital filter applies a different attenuation to each frequency and is characterized by its bandwidth, which is a measure of the range of frequencies retained in the filtered spectrum. Both high pass filtering and derivative spectrometry behave as digital filters, and their performances have been compared in the specialist literature dealing with signal handling [3, 4]. The mathematics will not be repeated here. The transfer function H is a measure of the attenuation applied to each frequency f and is given by

$$H = 1 - \cos^{2n}\left(\frac{\pi f}{2f_N}\right) \quad (1)$$

where f_N is the Nyquist frequency (half the sampling frequency) and n is the number of smooths applied to the subtracted spectrum.

In the limiting case, high pass digital filtering with a single smooth of the subtracted spectrum is equivalent to calculating the second derivative. It is apparent from equation (1) that increasing the number of smooths (n) increases H and therefore increases the bandwidth of the filter, retaining more of the low frequency components. This effect is shown in Fig. 1 for a spectrum of pseudoephedrine hydrochloride. By contrast, increasing the derivative order decreases the bandwidth of the retained signal. High pass digital filtering acts as a wide band high pass filter, whereas derivative spectrometry acts as a narrow band high pass filter.

There are two main consequences:

(1) Under some circumstances derivative spectrometry, because of the narrower frequency response, may be better at rejecting interferences than high pass digital filtering. This limitation of the filtering method may be overcome by increasing the sampling rate (i.e. decreasing the wavelength separation of the points) to allow high pass filtering to be used.

(2) Where both methods will remove interferences, the bandwidth of high pass filtering can be tuned by varying the number of smooths to reject low frequency interferences and yet have a better signal to noise ratio than derivative spectrometry.

The two techniques would therefore seem to be complementary.

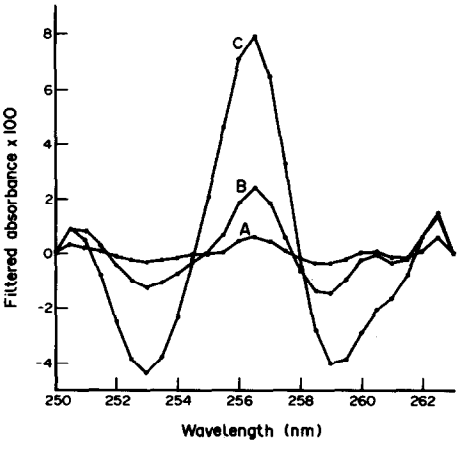

Figure 1
Effect of increasing the bandwidth of the digital filter for a spectrum of pseudoephedrine in 0.1 M hydrochloric acid. (A) 1 smooth (equivalent to minus the second derivative); (B) 5 smooths; (C) 50 smooths.

This paper gives a preliminary assessment of high pass filtering and aims to demonstrate its value for a typical problem in pharmaceutical analysis.

Experimental

High pass filtering was evaluated by applying it to a typical problem in pharmaceutical analysis, the assay of the decongestant pseudoephedrine hydrochloride in the presence of the antihistamine triprolidine hydrochloride. The drugs are formulated together in solid or liquid products in the proportions of 60 mg pseudoephedrine hydrochloride and 2.5 mg triprolidine hydrochloride per dose.

The conventional assay for these combinations in our laboratory is analogue second derivative spectrometry quantified by measuring the major deflection in the test and standard spectra. This method was used as a control.

Calculations

All calculations were carried out using Basic programs written for a 32K Commodore Pet model 8032 microcomputer with an 8050 disk drive and 4022 printer. The programs were split into two parts to permit the use of standard data on several occasions. One program writes a standard datafile to disk and the second calculates the results with a variable number of smooths and with the option to retain fixed endpoints or to lose the endpoints on each pass of the filter.

Spectrometry

Fixed wavelength measurements were made with a Pye–Unicam model SP8-200 UV-visible spectrophotometer. The same instrument was used for measuring the analogue second derivative spectra with the derivative accessory. All measurements were made with a 1 nm bandwidth and the solutions were maintained at 25°C.

Materials

Pseudoephedrine hydrochloride and triprolidine hydrochloride were of BP quality. Hydrochloric acid was analytical-reagent grade.

Procedure

Hydrochloric acid (0.1 M) was used as the solvent throughout and accurately prepared solutions containing 0.048% m/v pseudoephedrine hydrochloride were used as the standard. Linearity of response was measured by assaying solutions of pseudoephedrine hydrochloride containing up to 0.072% m/v, 150% of the assay concentration. The accuracy was determined by carrying out ten assays of a solution containing 0.048% m/v pseudoephedrine hydrochloride and 0.002% m/v triprolidine hydrochloride. A new standard solution was prepared on each occasion, and a parallel assay was carried out by second derivative UV spectrometry. The conditions for each method were as follows:

High pass digital filtering. Test and standard solutions were measured over the wavelength range 250–263 nm, step 0.5 nm. Fifteen smooths were carried out keeping the end points fixed.

Second derivative UV spectrometry. Test and standard solutions were scanned over the wavelength range 280–240 nm, recording each spectrum in triplicate. The mean deflection from the peak about 250 nm to the trough about 254 nm was determined.

Results and Discussion

UV spectra

Figure 2 shows the full UV spectra of pseudoephedrine hydrochloride and triprolidine hydrochloride. Spectra of the standard and test solutions from assay of the mixed pseudoephedrine and triprolidine solution are shown in Fig. 3. In this case the spectra are digitized over the wavelength range and interval used for the high pass filtering method. The triprolidine spectrum overlaps the full range of the pseudoephedrine spectrum and interferes considerably over the wavelength range selected for the assay. Although the interference appears to be linear over this restricted range, reference to Fig. 2 shows that the interference from triprolidine is a smooth curve. Some data treatment is essential to selectively determine pseudoephedrine. Figure 4 shows the filtered spectra (i.e. the subtracted result) and the residuals. The interference is almost

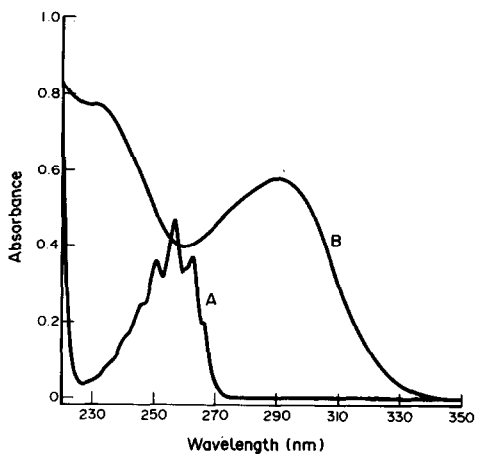

Figure 2
UV spectra of pseudoephedrine (A) and triprolidine (B).

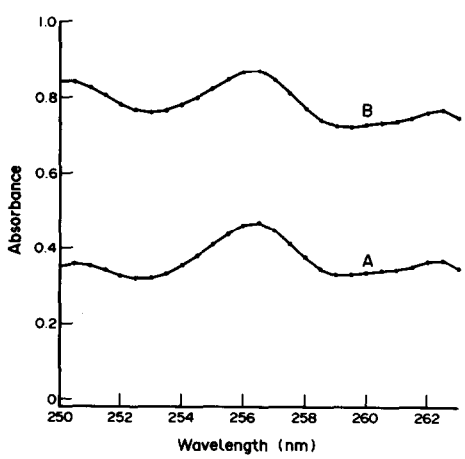

Figure 3
Digitized UV spectra of a standard solution (A) and a test solution (B) over the wavelength range used for the assay.

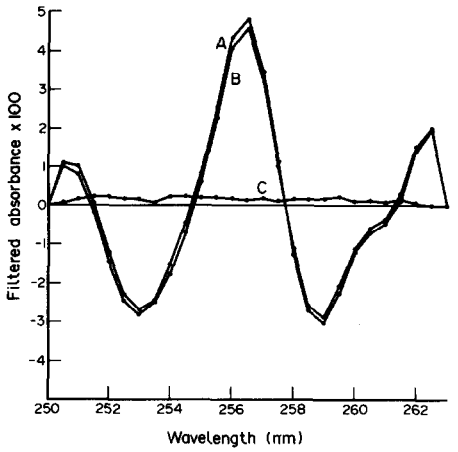

Figure 4
Filtered UV spectra of a standard solution (A) and a test solution (B) with the residuals (C).

completely removed. There is a slight systematic difference between the filtered test and standard spectra as shown by the positive value of the residuals. Because the filtered spectra have positive and negative lobes it is predicted on theoretical grounds that the residuals may all be of the same sign.

Validation of the digital filtering method

The response of standard pseudoephedrine solutions was linear over the range studied. The graph of response (as percentage of standard response) vs concentration (as percentage of standard concentration) has an intercept of −0.02, a slope of 1.0027, and a standard error of slope of 0.0025.

The accuracy and precision of the digital filtering method and of second derivative UV spectrometry are given in Table 1. The standard deviations were compared by an *F*-test and the results from each assay method compared by a paired *t*-test. Although high pass digital filtering appears to be more accurate and the second derivative method to be more precise, the differences are not significant ($P = 0.95$).

The high pass filtered results were also recalculated against a single standard solution so that the need to recalibrate on every occasion could be assessed. The results are compared in Table 2. As expected, the assay is more precise when calibrated on every occasion, though both sets of results are considered acceptable.

Examination of the residuals in Fig. 4 suggest that the digital filtering method compensates for a very small structured background absorption from triprolidine. This was confirmed by examination of a solution containing 0.002% m/v triprolidine which gave a response of 0.12% relative to a pseudoephedrine standard and showed a similar pattern of residuals.

It was considered necessary to show that the results were rugged to changes in wavelength range. A test solution containing pseudoephedrine and triprolidine was assayed against a standard, shifting the wavelength range by 0.5 nm in either direction. The results in Table 3 show a small reduction in the assay which is acceptable.

Table 4 shows the percentage of pseudoephedrine recovered from a solution containing triprolidine as a function of the number of smooths (the number of passes of

Table 1
Comparison of the accuracy and precision of high pass digital filtering and second derivative spectrometry for a solution containing pseudoephedrine and triprolidine

Method	Pseudoephedrine recovered (%)	Standard deviation
Digital filtering	99.48	0.758
Second derivative	99.14	0.469

Table 2
Results for the high pass digital filtering assay of a solution containing pseudoephedrine and triprolidine, comparing standardization on every occasion with the use of standard data obtained on a single occasion

Method	Pseudoephedrine recovered (%)	Standard deviation
Standardization on every occasion	99.48	0.758
Standardization on a single occasion	98.33	1.225

Table 3
Effect of shifting the wavelength range on the assay of a solution containing pseudoephedrine and triprolidine

Wavelength range (nm)	Pseudoephedrine recovered (%)
250–263	99.30
249.5–262.5	99.02
250.5–263.5	98.97

Table 4
Effect of the number of smooths on the assay of a solution containing pseudoephedrine and triprolidine

No. of smooths	Pseudoephedrine recovered (%)
5	99.02
10	99.25
15	99.30
20	99.29
30	99.13

the binomial filter used to smooth the subtracted spectrum). There is an optimum at 15, the number of smooths chosen for the assay, although the range of results is small.

The validation confirms that the high pass filtering assay is rugged and accurate, and would be suitable as a routine method for pharmaceutical analysis.

Conclusions

High pass digital filtering followed by a least squares fit of the standard to the test spectrum has been shown to be a useful technique for quantitative UV spectrometry in

the presence of interferences. An advantage of the method, which it shares with derivative spectrometry, is that the spectra of the interferences do not enter into the calculation. Good results were obtained for the assay of pseudoephedrine hydrochloride in the presence of triprolidine hydrochloride.

Further work is planned, including direct comparison with digital derivative spectrometry and the evaluation of peak to trough measurements. The latter will involve the use of bandpass filtering and interpolation.

High pass filtering may also be useful for other spectroscopic techniques such as fluorimetry and IR spectrometry.

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