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European Journal of Pharmaceutics and Biopharmaceutics 54 (2002) 337–341

European Journal of Pharmaceutics and Biopharmaceutics

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Research paper

Quantitative analysis of polymorphic mixtures of ranitidine hydrochloride by Raman spectroscopy and principal components analysis

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Received 22 April 2002; accepted in revised form 2 August 2002

Abstract

Ranitidine hydrochloride exists as two polymorphs, forms I and II, both of which are used to manufacture commercial tablets. Raman spectroscopy can be used to differentiate the two forms but univariate methods of quantitative analysis of one polymorph as an impurity in the other lack sensitivity. We have applied principal components analysis (PCA) of Raman spectra to binary mixtures of the two polymorphs and to binary mixtures prepared by adding one polymorph to powdered tablets of the other. Based on absorption measurements of seven spectral regions, it was found that >97% of the spectral variation was accounted for by three principal components. Quantitative calibration models generated by multiple linear regression predicted a detection limit and quantitation limit for either forms I or II in mixtures of the two of 0.6 and 1.8%, respectively. This study demonstrates that PCA of Raman spectroscopic data provides a sensitive method for the quantitative analysis of polymorphic impurities of drugs in commercial tablets with a quantitation limit of less than 2%.

Keywords: Ranitidine-HCl; Polymorphism; Raman spectroscopy; Principal components analysis; Quantitative analysis

1. Introduction

The polymorphic behaviour of drugs is a major concern of the pharmaceutical industry and ensuring an approved polymorph is present in a formulation is of crucial importance [1]. Ranitidine hydrochloride (*N*-[2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethyl]-*N*-methyl-2-nitro-l,l-ethenediamine hydrochloride) exists as two polymorphs, referred to as forms I and II, which differ in melting point and aqueous solubility but are therapeutically equivalent [2,3]. The two forms show somewhat different physical stabilities and undergo some interconversion on intensive grinding (forms I–II) [4] and possibly upon storage at elevated humidity (forms I–II) [5]. Since both polymorphs are used in commercial tablets, there is a need to monitor and quantify the presence of small amounts of one polymorph in formulations of the other.

A number of techniques have been used to characterise the two polymorphs of ranitidine hydrochloride including scanning electron microscopy [4], thermal analysis (DSC) [4], X-ray powder diffraction (XRPD) [6], IR spectroscopy (FTIR and DRIFTS) [7] and Raman spectroscopy [8]. X-ray diffractograms of ranitidine hydrochloride form I show characteristic high intensity peaks at 17.0°, 21.8° and 24.9° (2θ) whereas those of form II show strong peaks at 20.2° and 23.5° (2θ). The DRIFT spectra show that form I has a strong peak at 1551 cm^{-1} and form II a strong peak at 1046 cm^{-1}. The Raman spectra show characteristic peaks at 1208 cm^{-1} for form I and at 1185 cm^{-1} for form II.

Spectroscopic techniques are preferable to XRPD for the quantitation of polymorphic mixtures as they are more convenient and less time consuming [9]. Raman spectroscopy is particularly useful as it involves minimal sample preparation and, unlike IR spectroscopy, allows measurements to be made rapidly even in the presence of humidity. Taylor and Langklide [8] have investigated the use of Raman spectroscopy for the quantitative analysis of the polymorphic forms of ranitidine hydrochloride in tablets [8]. They showed that, using the characteristic peak at 1185 cm^{-1}, 5 and 10% of form II blended into tablets of form 1 (corresponding to 1.25–2.5% of the total weight of tablets) could be detected in the Raman spectra. The limit of quantitation was not measured but the authors concluded that 'by careful examination of the spectra, it is possible to monitor polymorph purity down to quite low levels for this system'.

In view of the fact that vibrational spectra of polymorphs

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0939-6411/02/$ - see front matter
PII: S0939-6411(02)00113-3

are usually very similar and may be lacking in characteristic peaks, multivariate analysis techniques (chemometrics) are often required [10,11]. Artificial neural networks (ANNs) have been applied but the technique is complex and requires large training sets to achieve a reasonable level of accuracy [7]. Principal components analysis (PCA) is more amenable and has been used to quantify a number of polymorphic mixtures [12]. Tudor et al. [12] applied it to near-infrared FT-Raman spectroscopic data of chlorpropamide polymorphs and Deeley et al. [13] performed similar investigations on polymorphic mixtures of cortisone acetate.

The aim of the present study was to investigate the application of Raman spectroscopy and PCA to the quantitative analysis of polymorphic mixtures of ranitidine hydrochloride, both as binary mixtures alone and in the presence of excipients. Of particular interest was to compare the limits of detection and quantification of this methodology with that of univariate analysis using the characteristic peaks of the two polymorphs.

2. Materials and methods

2.1. Materials

Samples of ranitidine hydrochloride form I were supplied by Dolorgiet Pharmaceuticals, Germany. Samples of ranitidine hydrochloride form II were prepared by recrystallising form I from isopropyl alcohol/hydrochloric acid. The two forms were characterised by XRPD as previously described [5] and shown to have similar particle size and morphology by optical microscopy. Tablets of ranitidine hydrochloride form I (150 mg) were supplied by Dolorgiet Pharmaceuticals, Germany. Zantac® tablets (150 mg) (GlaxoSmithKline) containing form II were obtained from a commercial supplier.

2.2. Sample preparation

Spectra were obtained of pure polymorphs and of binary geometric mixtures. Three different types of binary mixtures were prepared: mixtures of the two polymorphs with an interval of 10% (10–90%) and an interval of 1% (1–10 and 90–100%); mixtures of 1 to 5, 10, 20, 30, 40 and 50% ranitidine hydrochloride form I (percent of total weight) in tablets of form II; mixtures of 1 to 5, 10, 20, 30, 40 and 50% ranitidine hydrochloride form II (percent of total weight) in tablets of form I.

2.3. Raman spectroscopy

An argon-ion laser (Melles Griot, MAP-453-P) was used as an excitation source. Raman scattering was collected in a backscattering geometry, dispersed into a single-stage spectrograph (Spex 750M equipped with an 1800 groove/mm holographic grating) with a charge-coupled detector (CCD, Princeton Instruments 1152 EUV) controlled by a Princeton Instruments ST-130. CSMA v2.4 software (Princeton Instruments) was used to control the CCD. The excitation wavelength used was 457.9 nm with a laser power of approximately 15 mW at the sample. The Raman spectra were calibrated using krypton ion emission lines, which were checked using cyclohexane [14]. Rayleigh and Mie scattering from the sample was attenuated using a Notch filter (Kaiser Optical Systems Inc.) of appropriate wavelength. A polarisation scrambler was placed in front of the spectrograph entrance slit. All spectra were recorded in the range 200–1600 cm^{-1}. The acquisition time for each window was set at 3 min for all samples (5 s exposure; 36 measurements for each sample). Samples were placed in glass tubes (up to ~3 cm height) and scanned within 24 h of preparation. Seven peaks were chosen to provide the original data set for PCA based on peak heights (1589, 1552, 1249, 1210, 1188, 1164 and 1134 cm^{-1}) and peak areas (1600–1574, 1567–1541, 1288–1221, 1221–1194, 1194–1177, 1177–1150 and 1150–1117 cm^{-1}).

2.4. Principal components analysis

Spectra were analysed using GRAMS/32 (Galactic Industries Corp.) software. The data sets were subjected to PCA using Excel and the Minitab® v12.1 multivariate analysis statistical package. The principle of PCA is to reduce the dimensionality of a data set while retaining as much as

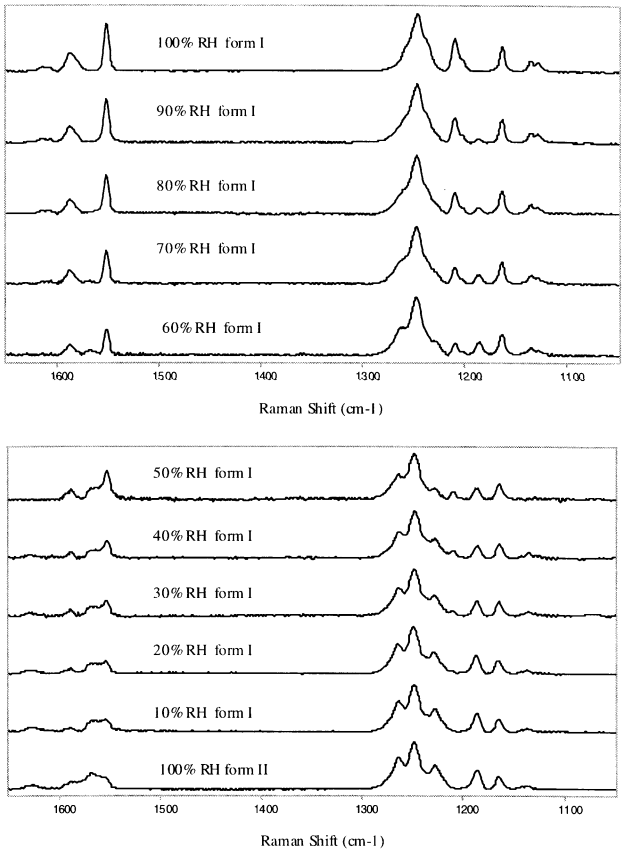

Fig. 1. Raman spectra of ranitidine hydrochloride forms I and II and of their binary mixtures.

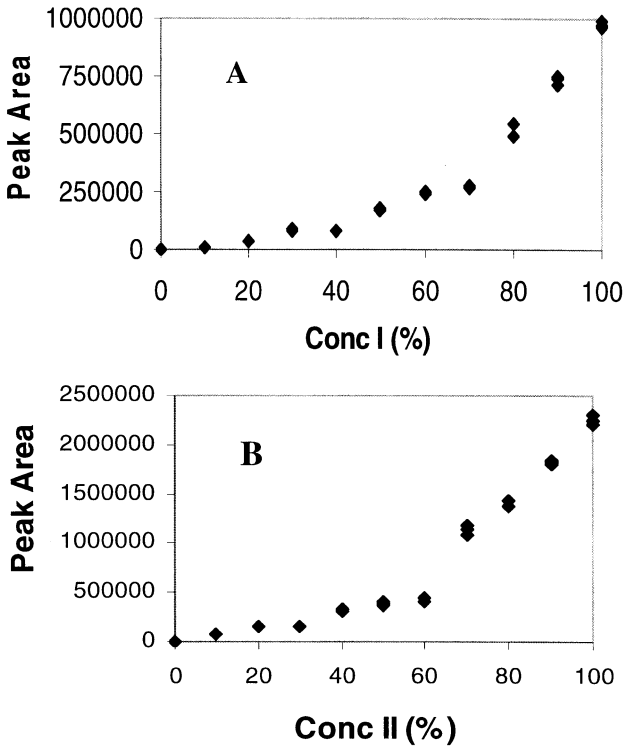

Fig. 2. Calibration curves of concentration (%) of ranitidine hydrochloride form I in binary mixtures with form II (A); and of form II in binary mixtures with form I (B) based on peak area at 1210 and 1188 cm^{-1}, respectively.

possible of the variation originally present [10]. This is achieved by transforming the spectral data to a new set of variables, the principal components (PCs), which are uncorrelated, and which are ordered so that the first few retain most of the variation present in all of the original variables. In this study, peak area and height of major peaks were used as the original variables to generate a correlation matrix containing a set of PCs equal in number to the number of peaks selected. Using the scree test, the PCs with relatively large eigenvalues were selected for regression analysis to provide quantitative prediction models of the composition of the binary mixtures. Linear regression of actual versus predicted concentration data provided 95% confidence intervals (CI) and prediction intervals (PI) [15]. The detection limit (DL) and quantitation limit (QL) of polymorphs in binary mixtures and of one polymorph in mixtures with tablets of the other were calculated from the standard deviation (σ) at the lowest concentration studied (1%) and the slope of the linear regression of the actual versus predicted concentration plot using $DL = 3.3\sigma/\text{slope}$ and $QL = 10\sigma/\text{slope}$ [16].

3. Results and discussion

The Raman spectra of ranitidine hydrochloride forms I and II and their binary mixtures are shown in Fig. 1. The Raman spectra of the pure forms are in good agreement with those published elsewhere [4,8]. In our hands, ranitidine hydrochloride form I shows a characteristic peak at 1210 cm^{-1}, whilst form II shows a characteristic peak at 1188 cm^{-1}. Both forms show absorption at 1589, 1552, 1249, 1164 and 1134 cm^{-1}.

In quantitation of binary mixtures using the characteristic peaks, calibration curves were found to be non-linear ($R^2 < 0.75$, Fig. 2). PCA based on the seven peaks showed that between 97% (for peak height) and 99% (for peak height) of all the spectral variation could be accounted for by a total of three PCs with proportions of 65% (PC1), 27% (PC2), and 7% (PC3) for peak area and 50% (PC1), 33% (PC2), and 14% (PC3) for peak height. The rest of the PCs (PC4–PC7) gave very small contributions (<0.7% for area, <1.3% for height) and were ignored.

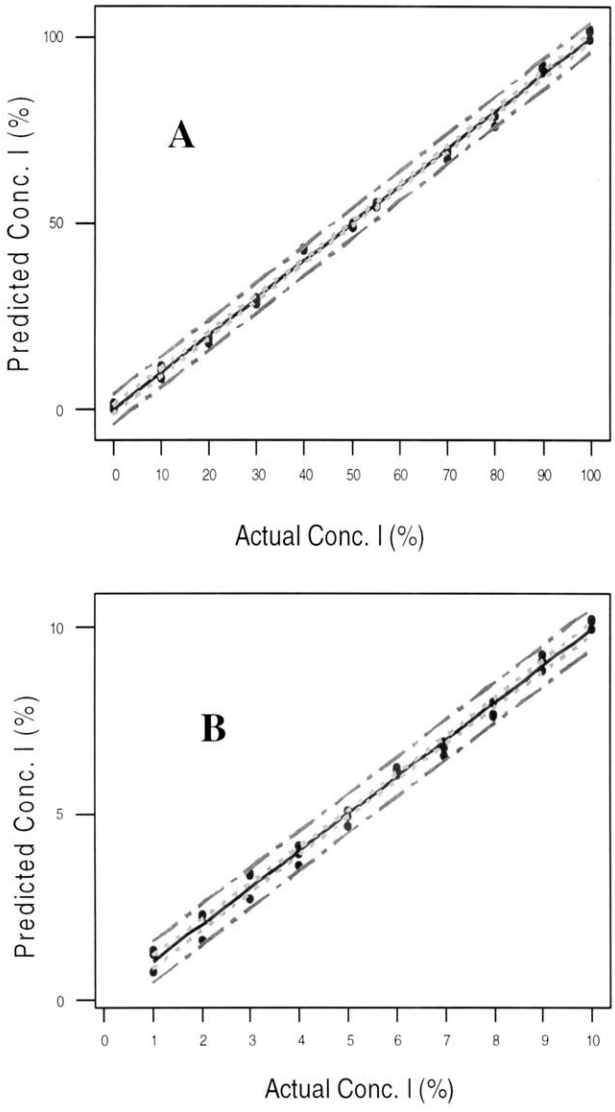

Fig. 3. Linear regressions of actual versus predicted concentration of ranitidine hydrochloride form I (%) in binary mixtures with form II over the entire range of binary mixtures (A); and at low levels (1–10%) (B) based on peak area (— regression line, ····· 95% CI lines; —·— 95% PI lines).

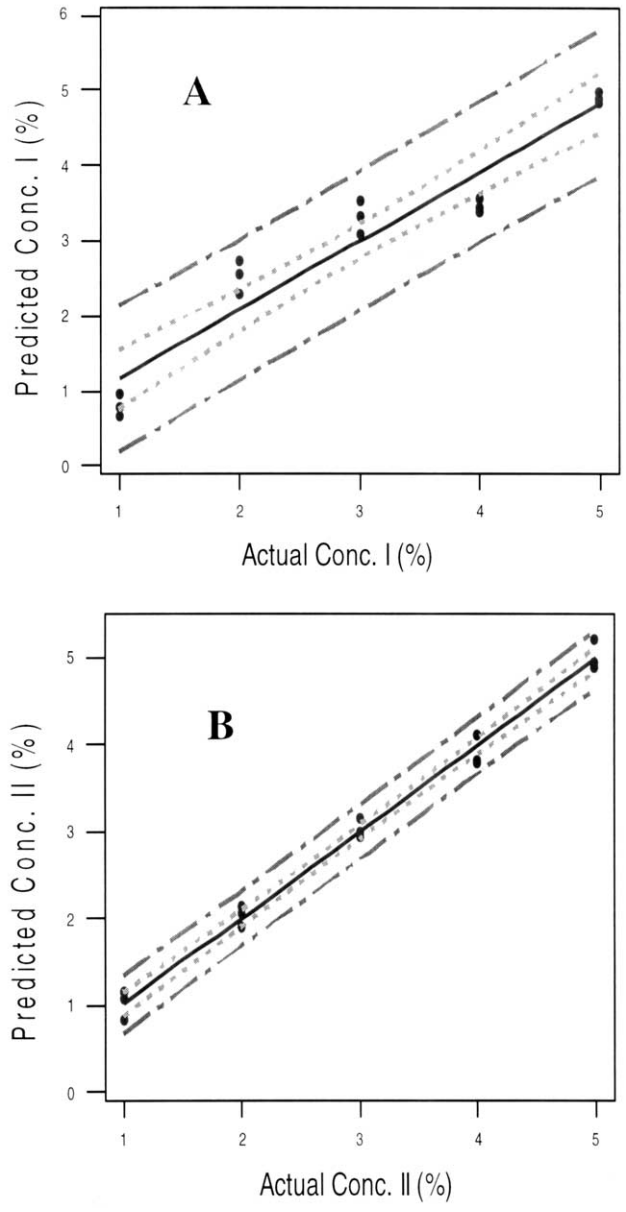

Fig. 4. Linear regressions of actual versus predicted concentration of ranitidine hydrochloride form I (1–5%) in tablets of form II (A); and of form II (1–5%) in tablets of form I (B) based on peak area (— regression line, ····· 95% CI lines; —·— 95% PI lines).

Multiple linear regression was carried out using concentration of form I as response and PC1, PC2 and PC3 as predictors. The regression equations based on peak area and height were % form I = 49.5 − 13.8 PCI + 8.56 PC2 + 1.41 PC3 ($R^2 = 0.989$) and % form I = 49.5 − 14.70 PC1 − 10.5 PC2 − 2.11 PC3 ($R^2 = 0.996$), respectively. The high correlation coefficients indicate good linear relationships. Linear regression of actual versus predicted concentration of ranitidine hydrochloride form I (1–10%) in binary mixtures with form II based on peak areas and heights gave regression lines with slopes consistently close to 1.0 and 95% CI and PI indicative of a high level of precision. Fig. 3A shows actual versus predicted concentration of forms I in II over the entire range of binary mixtures based on peak area. Fig. 3B shows the corresponding plot for low levels of forms I (1–10%) in II. Essentially similar data were obtained for peak height and for mixtures of forms II in I (data not shown).

As regards Raman spectra of commercial tablets, excipients did not appear to interfere in the wavenumber range examined as no additional peaks were noted. PCA of peak area and height data for mixtures of ranitidine hydrochloride form I in tablets of form II gave the regression equations: % form I = 25.8 −7.24 PCI − 0.975 PC2 − 1.32 PC3 ($R^2 = 0.998$) and % form I = 25.8 − 7.72 PC1 + 0.371 PC2 + 3.59 PC3 ($R^2 = 0.986$), respectively. PCA of corresponding data for ranitidine hydrochloride form II in tablets of form I gave the regression equations: % form II = 25.8 + 7.07 PC1 − 0.346 PC2 + 4.35 PC3 ($R^2 = 0.989$) and % form II = 25.8 − 7.64 PC1 + 2.00 PC2 − 1.61 PC3 ($R^2 = 0.990$), respectively. Linear regressions of actual versus predicted concentration of ranitidine hydrochloride form I (1—5%) in tablets of form II and of form II (1–5%) in tablets of form I based on peak area are shown in Figs. 4A,B, respectively.

The DL and QL of ranitidine hydrochloride forms I and II, both in binary mixtures and in mixtures with tablets of the other polymorph, were 0.6 and 1.8%, respectively. These values are smaller than those found by DRIFTS and XRPD using ANNs which gave DL and QL values for ranitidine hydrochloride form II in binary mixtures with form I of 1.5 and 5.2%, respectively [7].

4. Conclusion

This study demonstrates that PCA of Raman spectroscopic data provides a sensitive method for the quantitative analysis of polymorphic impurities in commercial tablets, with a quantitation limit of less than 2%. The results suggest Raman spectroscopy in combination with PCA may be useful as a screening method for the quality control of polymorphic drugs in solid dosage forms.

Acknowledgements

The support of the New Zealand Ministry of Foreign Affairs and Trade for an NZODA-Scholarship for DP is gratefully acknowledged.

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