Research Article

Drug Testing and Analysis

Received: 17 May 2010 Revised: 15 June 2010 Accepted: 15 June 2010 Published online in Wiley Online Library: 1 September 2010

(www.drugtestinganalysis.com) DOI 10.1002/dta.152

An experimental and computational study on the epimeric contribution to the infrared spectrum of budesonide

H. R. H. Ali,[a,b]* H. G. M. Edwards,[c] J. Kendrick,[d] T. Munshi,[c] and I. J. Scowen[c]

Budesonide is a mixture of 22R and 22S epimers. The epimeric content of budesonide was reported in both British and European pharmacopoeias to be within the range of 60–49/40–51 for R and S epimers, respectively. In this work, contribution of the two epimers to the overall infrared spectrum of budesonide has been investigated by quantum chemical calculations.

Keywords: budesonide; epimers; infrared; quantum chemical calculations; pharmaceuticals

Introduction

Budesonide is a second-generation glucocorticoid with low systemic absorption. It is used as an anti-inflammatory agent in the treatment of asthma, rhinitis, and inflammatory bowel disease.[1–3]

Structurally, budesonide is a 16α, 17α-acetal prepared by reaction of the 16α, 17α-dihydroxysteroid (16α-hydroxyprednisolone) with *n*-butyraldehyde. Due to the introduction of the alkyl chain at the C_{22} atom, budesonide is a mixture of two epimers (22*R* and 22*S*) (Figure 1).[4] Both epimers appear to have similar pharmacological effects; however, *in vitro* studies have suggested that the R epimer is two to three times more potent with respect to its anti-inflammatory effects.[5]

The pharmacopoeial monographs for budesonide appear in the British and European Pharmacopoeias [6, 7] which state that the *R*/*S* epimer ratio should be within the range of 60 ~ 49/40 ~ 51.

The crystal structures of R and S epimers are known and contained in the Cambridge Crystal Structure Database (CSD) with codes RHBUXP10 and SHBUXP10, respectively.

In recent years there has been increasing interest in the application of quantum chemical calculations for better understanding of the complex vibrational spectra of many pharmaceutical compounds.[8–15]

Our earlier study[8] has presented a comprehensive vibrational spectroscopic characterisation of budesonide considering only the 22S epimer. We report herein a detailed computational analysis of the effect of epimeric content of budesonide on its infrared (IR) spectrum in conjunction with powder X-ray diffractometry.

Experimental

Materials

A specimen of budesonide was obtained from Sigma Aldrich, Tokyo, Japan and used without further purification.

Powder X-ray diffractometry

Powder X-ray diffraction (PXRD) data were recorded with a Bruker D8 diffractometer with Cu-K$\alpha_{1,2}$ radiation (1.5418 Å) and a secondary curved graphite monochromator. The X-ray tube was operated at 40 kV and 30 mA. Samples were scanned in vertical Bragg-Brentano ($\theta/2\theta$) geometry (flat reflection mode) from 5–40° (2θ) using a 0.005° step width and a 1.5 s count time at each step. The receiving slit was 1° and the scattering slit was 0.2°. Data were analyzed with Bruker AXS Topas 2.1 software including a correction for axial divergence. Background coefficients, cell parameters, sample displacement error, and phase fractions were optimized parameters.

IR spectroscopy

The IR spectra were recorded as KBr discs (1 : 200) using a Digilab Scimitar Series spectrometer. The spectra were recorded in the range of 400–4000 cm^{-1} at 4 cm^{-1} spectral resolution with the accumulation of 512 spectral scans.

Raman spectroscopy

Raman spectra were recorded using a Raman microscope (Renishaw plc.) with 785 nm stabilized diode laser excitation. A 50x objective lens was used, giving a laser spot diameter of 2 µm at the sample. Spectra were obtained for one 20 s exposure of the CCD detector using the extended scanning mode of the instrument. Daily wavenumber calibration is required and is achieved by

* *Correspondence to: H. R. H. Ali, Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, University of Assiut, Assiut, 71526 Egypt. E-mail: hareha11374@gmail.com*

a *Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, University of Assiut, Assiut, 71526 Egypt*

b *Department of Chemistry, Faculty of Science, Gakushuin University, 1-5-1 Mejiro, Toshima-ku, Tokyo 171-8588 Japan*

c *Chemical and Forensic Sciences/University Analytical Centre, University of Bradford, Bradford, BD7 1DP UK*

d *Institute of Pharmaceutical Innovation, University of Bradford, Bradford, BD7 1DP UK*

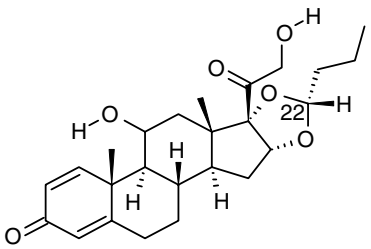

Figure 1. The chemical structure of epimer 22R of budesonide. The S epimer inverts the position of the proton and the propyl grouping on C-22.

recording the Raman spectrum of silicon (1 accumulation, 10 s for static mode). If necessary, an offset correction is performed to ensure that the position of the silicon band is 520.5 ± 0.1 cm^{-1}. The spectrometer was controlled by commercial instrument software (Renishaw WiRE2 Service Pack 8).

All vibrational spectra and PXRD patterns were exported to the Galactic SPC format using GRAMS AI (Galactic Industries, Salem, NH, USA, Version 8.0)

Computational analysis

Calculations were performed on the two molecules using the GAMESS-UK [16] and ORCA programs. [17] Geometry optimization was initially performed using a semi-empirical PM3 Hamiltonian and then further refined using BLYP density functional theory with a 6-31G* basis set.[17–19] IR and Raman spectra were calculated using the quasi-harmonic approximation, through diagonalization of the mass-weighted Hessian matrix. Intensities were calculated using the dipole-moment derivatives and the polarizability derivates of the normal modes.

Results and Discussion

Powder X-ray diffractometry (PXRD)

The composition of budesonide sample was investigated by PXRD. All peaks in the pattern coincided with theoretical patterns for the R- and S-epimer forms generated from single crystal studies (Figure 2).[4] Notably, no halo attributable to amorphous content in the sample was apparent. Peaks in the pattern were significantly broadened and pattern-fitting using the Rietveld approach indicated the presence of only two components underpinning the major peaks in the pattern. The broadening was hence attributed to the mixture of epimers in the sample. Quantitative Rietveld analysis, based on the single crystal structures, indicated the mixture to be composed of epimers in the ratio R : S of 51 : 49. The two epimers of budesonide are known to form solid solutions in the mixtures over the composition range 0–95%.[4] Inclusion of both epimers in the same crystal is likely to induce significant disorder which manifests as peak broadening in the diffraction pattern[20] (Figure 2).

Computational and vibrational studies

The two epimers of budesonide were investigated (Figure 1). For both epimers full geometry optimization was carried out, followed by the determination of the predicted vibrational frequencies. The hybrid BLYP density function was used throughout this work because it shows good performance with a minimum error.[21]

The predicted IR and Raman spectra of the two epimers 22R and 22S over the regions 1000–1800 cm^{-1} and 100–1100 cm^{-1} are compared in Figures 3, 4 and 6, 7, respectively. The recorded IR and Raman spectra of budesonide in the region 100–1800 cm^{-1} are presented in Figures 5 and 8, respectively. The simulated spectra were generated using the computed frequencies and intensities

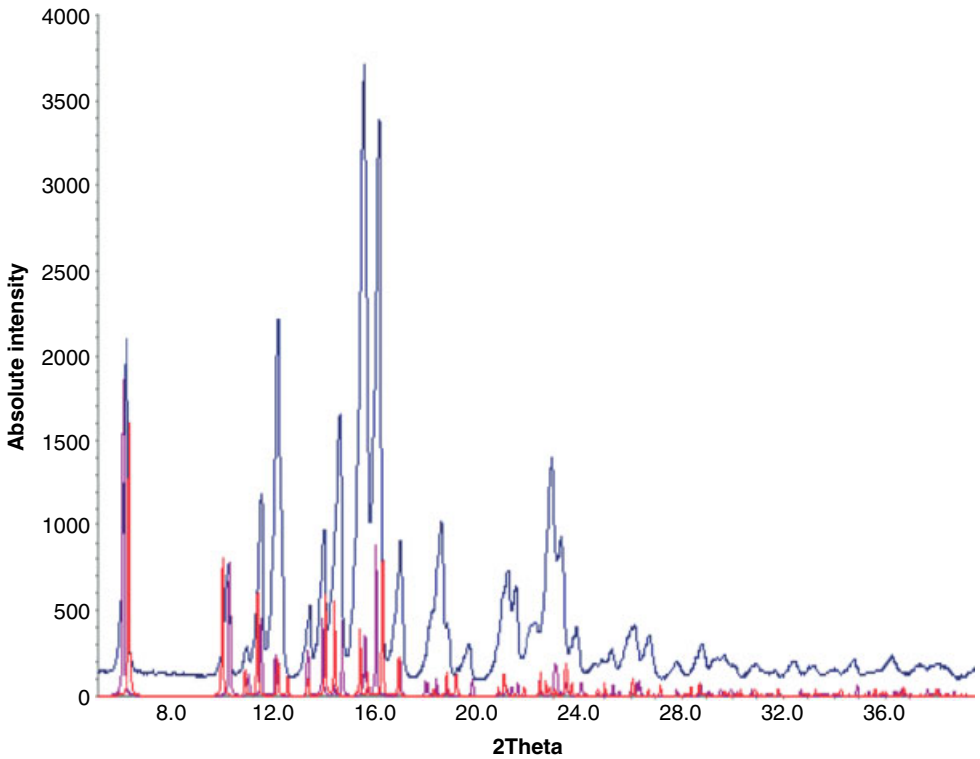

Figure 2. PXRD pattern of budesonide epimers (the red is 22R and the blue is 22S).

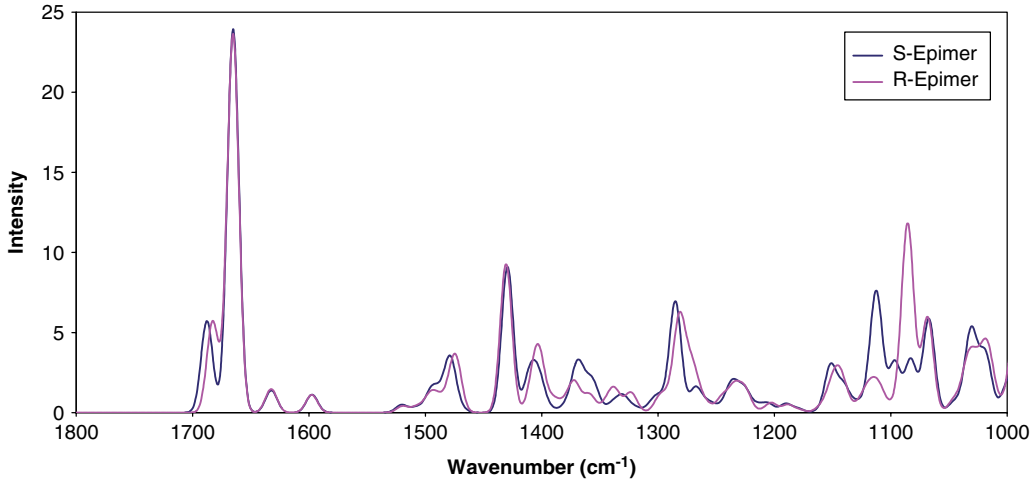

Figure 3. Comparison of the predicted IR spectra of 22R and 22S epimers in the wavenumber range of 1000–1800 cm^{-1}.

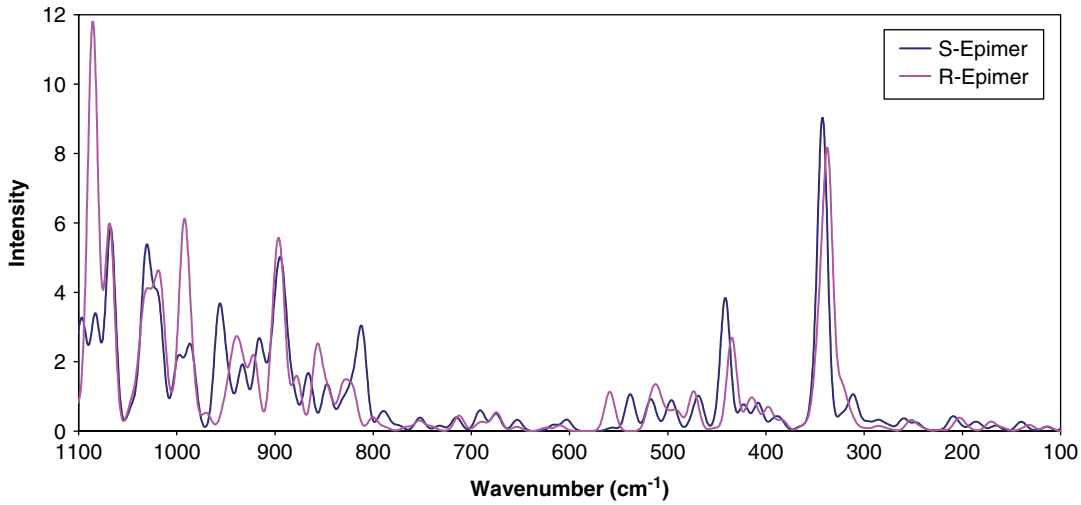

Figure 4. Comparison of the predicted IR spectra of 22R and 22S epimers in the wavenumber range of 100–1100 cm^{-1}.

and applying a Gaussian broadening to each transition of 5 cm^{-1} and 2 cm^{-1} in the IR and Raman spectra, respectively.

The shifts observed in the predicted IR spectra of the two epimers are more apparent than those observed in the predicted Raman spectra and are summarized in comparison with the observed IR bands of budesonide in Table 1. It can be noted that the observed IR spectral features comprise the predicted IR spectral features of both 22R and 22S epimers. The small shift in frequencies between the two epimers reflects the small influence from the epimeric content of budesonide on its IR vibrational modes. The quantum chemical calculations are indicating that the most intense IR spectral features of budesonide in the region 1600–1800 cm^{-1} are not affected by its epimeric content (Figure 3). The largest changes in the predicted IR spectra for both epimers are seen in the region 950–1300 cm^{-1}. For the R epimer, Figures 3 and 4 show two strong calculated absorptions at 1086 and 993 cm^{-1} (associated with the dioxolane ring) and a medium absorption at 1282 cm^{-1} (associated with carbon atoms of the acyloin group attached to the dioxolane ring) which are observed experimentally at 1092, 982 and 1292 cm^{-1} (Figure 5), respectively. The S epimer, in turn, has three medium calculated absorptions (Figures 3 and 4) at 1285 cm^{-1} (associated with the acyloin group attached to the dioxolane ring), 1112 cm^{-1} (associated with the propane chain attached to the dioxolane ring) and 957 cm^{-1} (associated with the dioxolane ring) which are recorded at 1292, 1110, and 956 cm^{-1} (Figure 5), respectively. These data (Table 1) show a good overall agreement with the powder X-ray diffractometric analysis carried out in this work and the reported compendial monographs[6, 7] of budesonide about its epimeric content.

Conclusions

The predicted IR vibrational modes of 22R and 22S epimers of budesonide provided insight into its solid-state structural analysis. In particular, it was possible to indicate that budesonide specimens in the solid-state are a mixture of the two epimers which agrees with its reported compendial monographs and the powder X-ray diffractometric analysis carried out in this work. It is worth to emphasize that this vibrational spectroscopic approach has yielded valuable complementary information to the powder X-ray analysis. Quantum chemical calculations offer great potential to improve understanding of the structural properties of solid-state pharmaceuticals and these are of particular importance in the

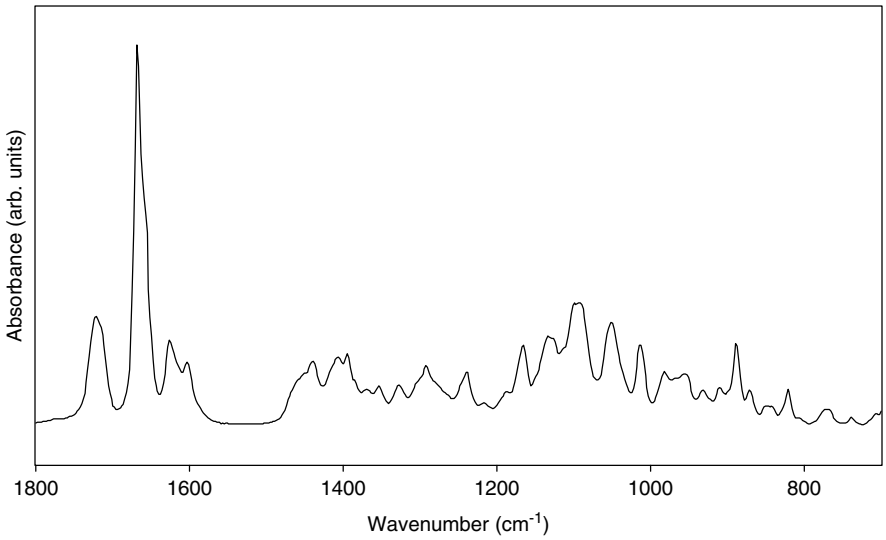

Figure 5. The recorded IR spectrum of budesonide in the wavenumber range of 100–1800 cm^{-1}.

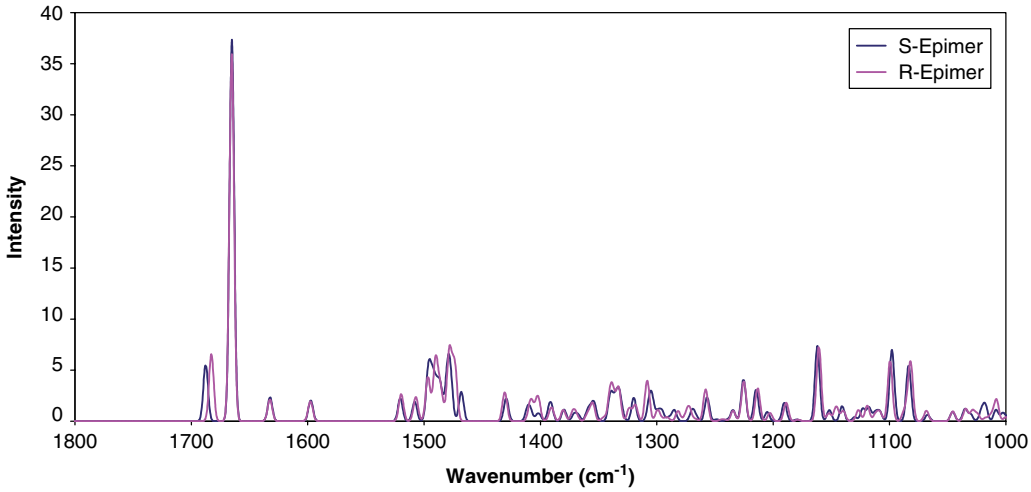

Figure 6. Comparison of the predicted Raman spectra of 22R and 22S epimers in the wavenumber range of 1000–1800 cm^{-1}.

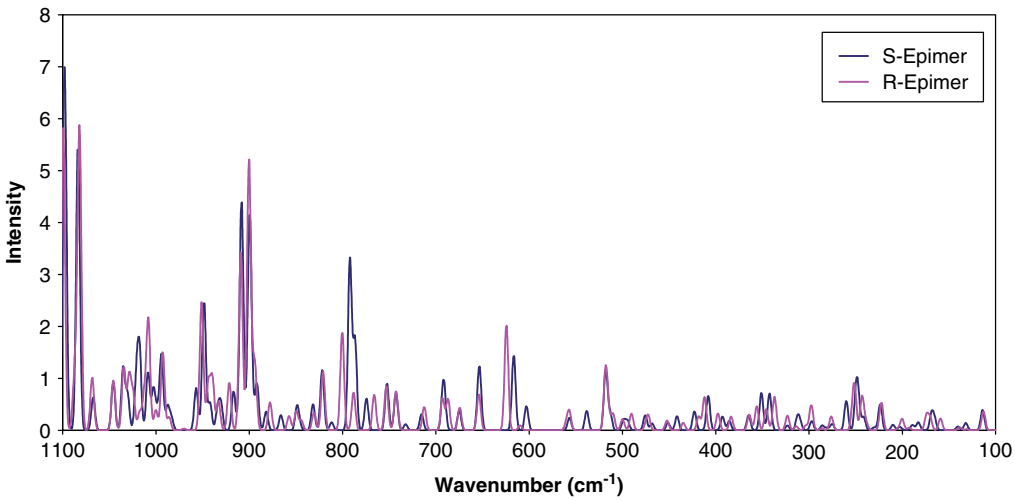

Figure 7. Comparison of the predicted Raman spectra of 22R and 22S epimers in the wavenumber range of 100–1100 cm^{-1}.

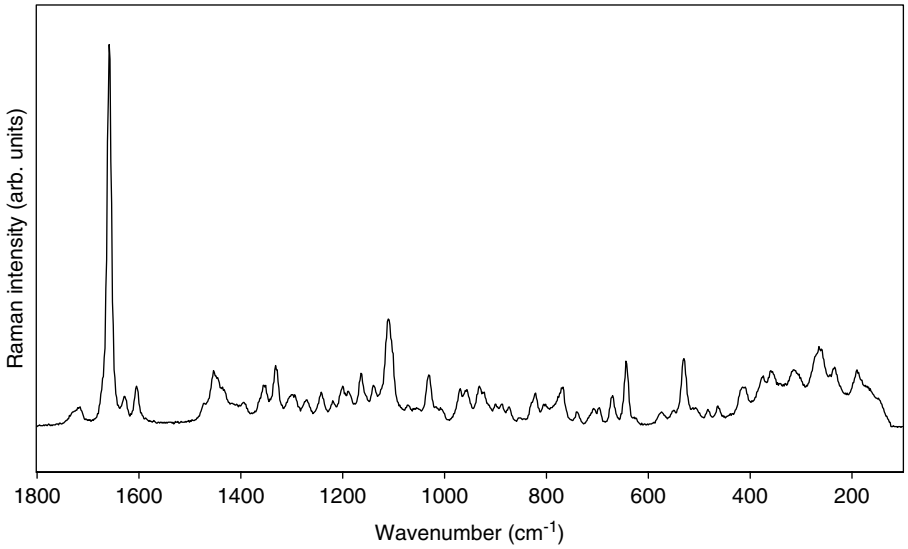

Figure 8. The recorded Raman spectrum of budesonide in the wavenumber range of 100–1800 cm^{-1}.

Table 1. The key predicted IR spectral features of 22R and 22S epimers of budesonide in comparison with its IR observed bands in the range of 100–1800 cm^{-1}

22R	22S	Observed
1683 mw	1687 mw	1721 s
1404 w	1403 w	1407 ms
1375 w	1367 w	1369 mw
1325 w	1330 w	1327 mw
1282 m	1285 m	1292 mw
1147 w	1152 w	1166 ms
1117 w	1112 m	1110 m
1086 s	1097 w	1092 m
1020 ms	1031 ms	1013 m
993 s	987 mw	982 mw
941 mw	957 m	956 mw
857 m	866 w	872 mw
830 w	821 m	821 m

absence of more detailed structural information, as in the case of many other pharmaceutical systems.

References

[1] R. Hamedani, R. D. Feldman, B. G. Feagan, *Aliment. Pharmacol. Ther.* **1997**, *11*, 98.
[2] K. M. Hvizdos, B. Jarvis, *Drugs* **2000**, *60*, 1141.
[3] C. M. Spencer, D. McTavish, *Drugs* **1995**, *50*, 854.
[4] J. Albertsson, A. Oskarsson, C. Svensson, *Acta Crystallogr. Sect. B: Struct. Sci.* **1978**, *34*, 3027.
[5] R. Brattsand, A. Thalen, K. Roempke, *et al*., *J. Steroid Biochem.* **1982**, *16*, 779.
[6] *British Pharmacopoeia*, The Stationery Office on behalf of the Medicines and Health Care Products Regulatory Agency (MHRA): London, **2009**.
[7] *European Pharmacopoeia*, The European Department for the Quality of Medicines within the Council of Europe: Strasbourg, **2005**.
[8] H. R. H. Ali, H. G. M. Edwards, J. Kendrick, *et al*., *J. Raman Spectros.* **2007**, *38*, 903.
[9] H. R. H. Ali, H. G. M. Edwards, J. Kendrick, *et al*., *Spectrochim. Acta Part A* **2009**, *72*, 244.
[10] H. R. H. Ali, H. G. M. Edwards, J. Kendrick, *et al*., *Drug Test. Anal.* **2009**, *1*, 51.
[11] H. R. H. Ali, H. G. M. Edwards, J. Kendrick, *et al*., *Spectrochim. Acta, Part A* **2009**, *72*, 715.
[12] H. R. H. Ali, H. G. M. Edwards, J. Kendrick, *et al*., *Drug Test. Anal.* **2009**, *1*, 73.
[13] K. C. Gordon, C. M. McGoverin, C. J. Strachan, *et al*., *J. Pharm. Pharmacol.* **2007**, *59*, 271.
[14] L. Moroni, C. Gellini, M. M. Miranda, *et al*., *J. Raman Spectros.* **2008**, *39*, 276.
[15] A. Schmidt, A. S. Lindner, J. Casado, *et al*., *J. Raman Spectros.* **2007**, *38*, 1500.
[16] M. F. Guest, I. J. Bush, H. J. J. Van Dam, *et al*., The GAMESS-UK electronic structure package: algorithms, developments and applications, **2005**.
[17] F. Neese, ORCA – An ab initio, Density Functional and Semiempirical Program Package, Version 2.4. Max-Planck-Institut fuer Bioanorganische Chemie, Muelheim and der Ruhr, **2004**.
[18] A. D. Becke, *Phys. Rev. A* **1988**, *38*, 3098.
[19] C. Lee, W. Yang, R. G. Parr, *Phys. Rev. B* **1988**, *37*, 785.
[20] R.W. James, *The Optical Principles of the Diffraction of X-Rays*, Ox Bow Press: Woodbridge, USA, **1982**.
[21] F. Jensen, *Introduction to Computational Chemistry*, 2nd Edn, John Wiley & Sons, Ltd: Chichester, **2007**.