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A high-performance liquid chromatography and nuclear magnetic resonance spectroscopy-based analysis of commercially available praziquantel tablets

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Abstract

The amount of active ingredient in 20 commercially sourced batches of praziquantel (PZQ) tablets was determined using a high-performance liquid chromatography–ultraviolet (HPLC–UV) assay in conjunction with an anthentic, lot of PZQ powder. The general composition of each batch of tablets was also examined by means of ^{1}H nuclear magnetic resonance (NMR) spectroscopy and the NMR data were subjected to pattern recognition analysis by means of principal component analysis. The HPLC–UV results showed that each batch of PZQ tablets contained approximately the required amount of PZQ (600 mg per tablet). The NMR analysis showed a high degree of compositional variation between manufacturers, which caused by variation in excipients, along with some batch-to-batch variation in the tablets from a single manufacturer. Additionally, the PZQ tablets from one manufacturer were found to have an extra component (methyl-4-hydroxybenzoate) that was not detected in the other preparations.

Keywords: High-performance liquid chromatography; Nuclear magnetic resonance spectroscopy; Praziquantel; Principal component analysis; Quality control; Schistosomiasis

1. Introduction

Praziquantel (PZQ) is the current drug of choice for the treatment and control of schistosomiasis [1–4] and the majority of the food-borne trematode infections [5,6]. PZQ has a good safety and therapeutic profile and has been widely used in community-based schistosomiasis control programmes. For example, in China and Egypt alone, more than 100 million doses have been administered over the past two decades [4,7]. The price of PZQ has plummeted since 1990 and a 2001 World Health Assembly resolution urges member states to regularly administer PZQ to school-aged children and other high-risk groups in areas that are highly endemic for schistosomiasis [1]. A global partnership has been created and the Schistosomiasis Control Initiative (SCI) is supporting several countries in sub-Saharan Africa with national control programmes emphasising PZQ-based chemotherapy [2,8].

These developments are welcomed but the price of PZQ still varies between schistosome-endemic countries and concern has been raised with regard to the PZQ content of tablets, lack of information on shelf life, and inter-manufacturer variation in physico-chemical properties and excipients [9]. Since the use of PZQ preparations with lower efficacy may contribute to the development of drug resistance, a 'Concerted Action on Praziquantel' initiative was launched in the late 1990s to collect and perform quantitative and qualitative analysis on PZQ from different endemic settings and to monitor trends over time [9–11]. Findings reported at the 4th meeting of the 'Concerted Action

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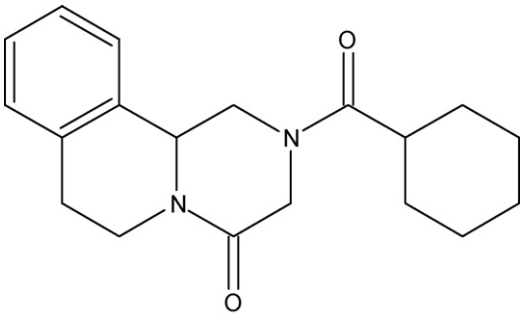

Fig. 1. Chemical structure of PZQ.

on Praziquantel' in 2001 also indicated that, although products from 3 of the 19 manufacturers investigated were slightly less than optimal in terms of solubility and impurities, the quality of all 19 products was acceptable in terms of purity and amount of active ingredient in each tablet tested [12]. In contrast, two samples of one brand of tablet collected previously in the Sudan were found to be counterfeit and to contain no active ingredient [13].

The purity of a sample of PZQ drug material (Fig. 1) can be assessed using a high performance liquid chromatography–ultraviolet (HPLC–UV) absorbance assay as described in the relevant European Pharmacopoeia 2005 official monograph [14]. According to the European Pharmacopoeia, the total peak area at 210 nm from all impurities should not exceed 0.5% of the area of the PZQ peak at that wavelength. However, the European Pharmacopoeia does not specify a method for PZQ tablets and it appears that at least some workers analysing PZQ tablets have based their approach on the drug material method. Whilst that is a perfectly reasonable approach, it must be recognised that some of the additional formulated tablet components might show up in the HPLC analysis and care must be taken that they are not wrongly identified as impurities.

In the present work, we have used an adaptation of the European Pharmacopoeia HPLC–UV method for PZQ drug material to estimate the PZQ content of 20 batches of PZQ tablets supplied by four different manufacturers. ^{1}H nuclear magnetic resonance (NMR) spectroscopy, together with multivariate data analysis techniques, has previously been used to assess inter-batch variations in the chemical composition of drugs and traditional herbal medicines including feverfew, chamomile flowers, St John's Wort, ginseng and artemisinin derivatives [15–19]. We have also used high-resolution ^{1}H NMR spectroscopy in combination with pattern recognition analysis to examine variation in the overall composition of the PZQ tablets. Whilst the ^{1}H NMR method employed is, like the HPLC method, only suitable for analysing soluble components, it has the advantage that it is an almost universal detector for organic compounds and also provides structural information on the compounds detected.

2. Materials and methods

2.1. PZQ tablets

The 20 PZQ batches were obtained from four different companies. Batches 1–9 were supplied by Pharmchem International Ltd. (Harrow Middlesex, UK; nos. 102, 106, 40719, 40720, 40723, 40770, 40793a, 40793b and 40806). Batches 10–18 were from Med Pharm and supplied by Cipla Ltd. (Patalganga, India; nos. K40769, K40794, K40831, K41052, K41080, K41082, K41099, K41100 and G54020). Batch 19 was obtained from Tanzania Pharmaceutical Industries Ltd. (Arusha, Tanzania; no. RJ02), and batch 20 from Shelys Pharmaceutical Ltd. (Dar es Salaam, Tanzania; no. B.N. RD 001).

2.2. Determination of PZQ content of tablets by HPLC–UV

The column used was a TMC-Pack FL-ODS, 50 mm × 4.6 mm × 5 μm C18 column (Yamamura Chemicals Lab Co., Europe GmbH), which was maintained at an oven temperature of 30 °C. A mobile phase gradient (acetonitrile:water from 40:60 to 80:20 v/v) was applied over a 10 min run with a flow rate of 1.0 ml/min and UV detection at 262.5 nm. Here a slightly different wavelength was used compared to the European Pharmacopoeia since highest absorption for PZQ was at 262.5 nm.

Authentic PZQ (98.9%), purchased from Sigma was used for the response calibration with six calibration solutions, having PZQ concentrations in the range of 0.5–5 mg/ml in 50:50 (v/v) mixture of water and acetonitrile and a 100 μl aliquot of each solution manually injected onto the column.

For each batch, 10 PZQ tablets were taken at random and weighed as one lot prior to crushing and homogenisation. Then, three accurately measured amounts, in the range of 15–20 mg, of the ground powder were each dissolved as far as possible in 4.5 ml of a water–acetonitrile mixture (50:50 v/v) with any volume change assumed to be insignificant. Following agitation, 1 ml of each suspension was removed and centrifuged in a 1.5 ml microcentrifuge tube at 10,000 rpm for 2 min. The clear supernatant was removed for HPLC analysis (three replicate 100 μl injections) and its PZQ content determined by reference to the absorbance values obtained for the calibration standards. The average PZQ content of the relevant tablets was then calculated.

2.3. ^{1}H NMR spectroscopic analysis and pattern recognition of overall tablet composition

Three samples were analysed for each batch of tablets. In each case, 20 mg of the powdered material was mixed with 700 μl D_2O:acetonitrile-d_3 solvent (5:1 v/v) containing 0.05% (w/v) sodium 3-(trimethylsilyl) 2,2,3,3-d_4 propionate (TSP). The suspension was centrifuged at 10,000 rpm for 2 min and the clear supernatant transferred to a 5 mm NMR tube.

^{1}H NMR spectra were recorded on a Bruker DRX 600 NMR spectrometer (Bruker; Rheinstetten, Germany), equipped with a Bruker 5 mm TXI probe, operating at 600.13 MHz for ^{1}H NMR. A standard 1-dimensional (1D) presaturation pulse sequence (D1-90-t_1-90-t_m-90-acq) was employed to achieve water suppression [20]. The inter-pulse delay t_1 was 3 μs and the mixing time t_m was 100 ms. A weak irradiation was applied at the water resonance frequency during both the mixing time and the recycle delay, D1, of 2 s. The 90° pulse length was 10.5 μs and a total of 32 scans were collected into 32k data points with a spectral width of 20 ppm. The free induction decays were multiplied by an

exponential function corresponding to a 0.3 Hz line-broadening prior to Fourier transformation and the resulting spectra were manually corrected for phase and baseline distortions in XWIN-NMR (version 3.6 Bruker Analytik; Rheinstetten, Germany). The spectra, over the range δ 0.2–10.0 ppm were reduced using AMIX (Bruker Analytik) to 245 consecutive 0.04 ppm-wide regions and the signal intensity in each region integrated. The region δ 4.30–4.86 containing the residual water signals was removed and the remainder of each spectrum normalized to a constant total integral prior to pattern recognition analysis.

Principal component analysis (PCA) of the mean-centred intensity data values was performed in the Simca-P software (version 10.0, Umetrics; Umeå, Sweden). PCA is mathematic way to reduce complexity of multivariate datasets to facilitate visualization of inherent patterns in the data. Each principal component (PC) is a linear combination of variables whereby each successive PC explains the maximum amount of variance possible, not accounted for by the previous PCs and each PC is orthogonal to the other PCs. Data were visualised by plotting the PC scores, where each point on the scores plot represents an individual sample, and the PC loadings, where each point represents one spectral region. The scores and loadings plots are complementary. Differences between samples can be detected in the score plots whereas spectral regions responsible for the differences can be viewed in the corresponding loading plots [21].

2.4. Identification of the para-disubstituted aromatic compound in the Shelys product

This compound was isolated by flash chromatography (acetonitrile, silica) and examined by ^{1}H NMR spectroscopy and by ultra-performance liquid chromatography–mass spectrometry (UPLC–MS). Candidate compounds (4-methoxybenzoic acid and methyl-4-hydroxybenzoate) were then separately added to the isolate and further ^{1}H NMR spectra recorded. Having, thereby, identified the isolated compound as methyl-4-hydroxybenzoate, this compound was then added to an extract of the Shelys product and a further ^{1}H NMR spectrum recorded. HPLC–UV chromatograms were also recorded from an extract of the Shelys product before and after addition of methyl-4-hydroxybenzoate.

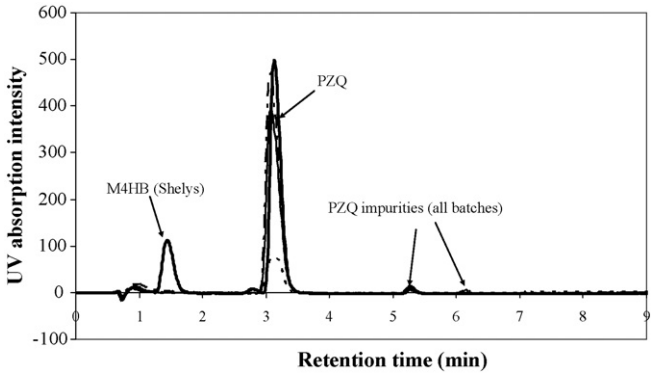

Fig. 2. HPLC–UV chromatograms for an authentic sample of PZQ drug material (dotted line) and for extracts of PZQ tablets from various manufacturers [Pharmchem International Ltd. (dotted-dashed line), Tanzania Pharmaceutical Industries Ltd. (thin line) and Shelys Pharmaceutical Ltd. (solid line)]. M4HB denotes methyl-4-hydroxybenzoate.

3. Results and discussion

3.1. HPLC–UV analysis of PZQ batches

The chromatograms generated for each of the 20 batches of PZQ investigated here contained several impurities, which was to be expected since pharmaceutical grade PZQ is known to contain three impurities, as mentioned earlier and as depicted in

Table 1
Quantitative results obtained from HPLC–UV analysis of 20 batches of PZQ tablets obtained from four different manufacturers

Lot	Manufacturer	Mean tablet mass (mg)	Estimated mass of PZQ per tablet in mg ± standard deviation[a]
1	Pharmchem International Ltd.	935.9	582.9 ± 6.2
2	Pharmchem International Ltd.	974.6	611.6 ± 6.2
3	Pharmchem International Ltd.	950.4	593.5 ± 17.0
4	Pharmchem International Ltd.	955.4	594.3 ± 2.8
5	Pharmchem International Ltd.	975.5	596.0 ± 3.1
6	Pharmchem International Ltd.	974.7	609.4 ± 4.9
7	Pharmchem International Ltd.	964.5	614.7 ± 10.1
8	Pharmchem International Ltd.	970.1	604.0 ± 8.4
9	Pharmchem International Ltd.	968.8	607.9 ± 4.3
10	Med Pharm	950.6	597.5 ± 4.8
11	Med Pharm	950.0	589.9 ± 4.3
12	Med Pharm	952.9	590.9 ± 11.1
13	Med Pharm	946.9	597.8 ± 2.5
14	Med Pharm	947.6	589.2 ± 14.1
15	Med Pharm	961.4	584.5 ± 18.5
16	Med Pharm	956.1	598.4 ± 8.0
17	Med Pharm	963.7	617.0 ± 9.7
18	Med Pharm	966.2	617.5 ± 3.8
19	Tanzania Pharmaceutical Industries Ltd.	904.5	619.3 ± 1.6
20	Shelys Pharmaceutical Ltd.	944.4	606.5 ± 7.8

[a] The standard deviation is based on three samples per batch and three injections per sample.

Fig. 2. Peaks at retention times 5.3 and 6.2 min are unidentified impurities and present in all the batches of tablets as well as PZQ drug material. However, our analysis revealed that the PZQ batch supplied by Shelys Pharmaceutical Ltd. contained an extra component that was not observed in the samples from the other manufacturers (Fig. 2) with the integration for that component being 22% of the integral of the PZQ peak. This is consistent with previous analyses of products from this source, as discussed during the 4th meeting of the 'Concerted Action on Praziquantel' [12].

The calibration for PZQ was found to be linear over the range of 0.54–4.92 mg/ml ($R^2 = 0.99$) and Table 1 shows the estimated PZQ content of each batch of the tablet. Our analysis indicates that all 20 batches investigated were broadly consistent with the declared amount of active ingredient (i.e. 600 mg per tablet) [12]. In fact, in all batches the error margin on the declared PZQ content appeared to be within 5% and in 15 batches (75% of all samples) the PZQ content appeared to be within 2.5% of the declared amount.

3.2. ^{1}H NMR spectroscopy and PCA of NMR data

PCA was performed on the ^{1}H NMR spectra acquired from the 20 batches of PZQ and a scores plot generated from the PCA is shown in Fig. 3. The plot demonstrates acceptable analytical performance with the three replicate samples for each batch being clustered together. As can be seen, six main clusters were observed in the scores plot of the first two PCs, which contained 98% of the total variance in the mean-centred data set. Three of these clusters correspond to batches obtained from Pharmchem International Ltd. with the remaining three clusters attributable to samples from the other three manufacturers. Thus, the tablets obtained from Med Pharm (and provided by Cipla Ltd.) showed greater batch-to-batch consistency than the tablets from Pharmchem International Ltd. where the observed batch-to-batch variation is assumed to be due to changes in drug excipients. The variation along the first PC was mainly due to resonances in the δ 3.5–3.9 region.

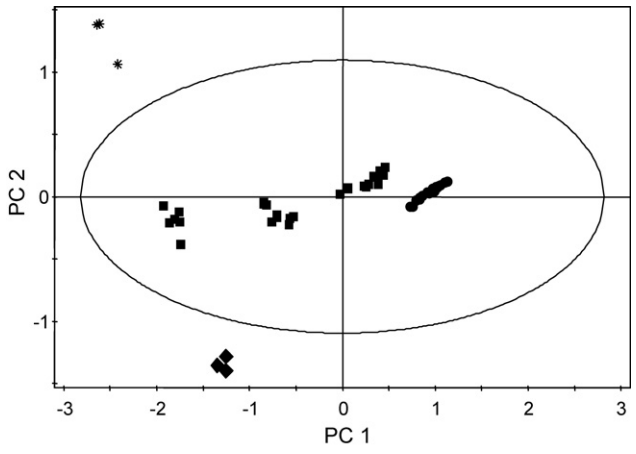

Fig. 3. Scores plot from PCA of ^{1}H NMR spectra of extracts of powdered PZQ tablets with three separate NMR analyses for each lot. Dot: Med Pharm; Box: Pharmchem International Ltd.; diamond: Shelys Pharmaceutical Ltd.; star: Tanzania Pharmaceutical Industries Ltd.

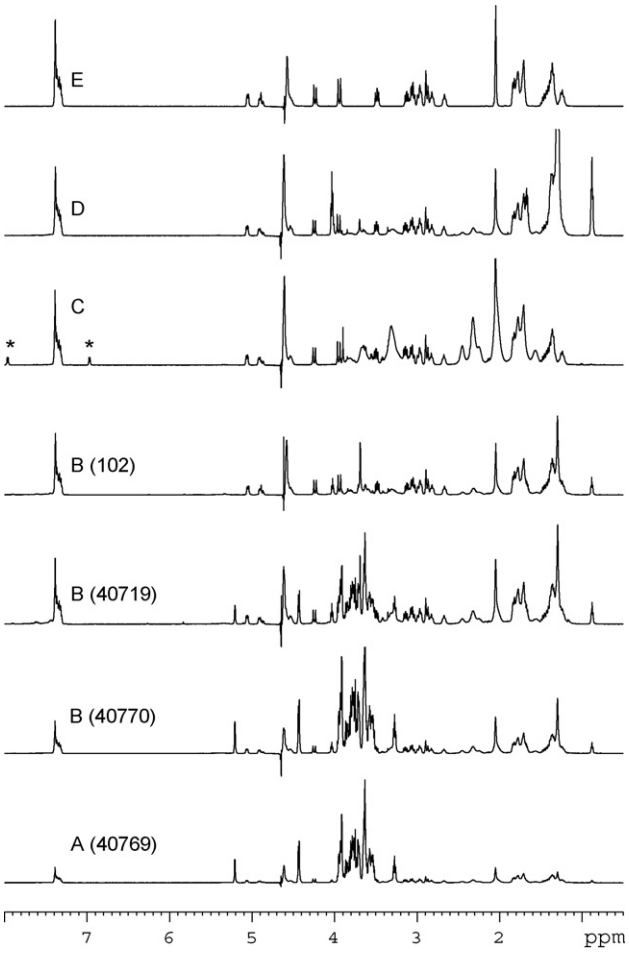

Fig. 4. 600 MHz ^{1}H NMR spectra representative of each of the clusters in Fig. 3. Key: (A) Med Pharm; (B) Pharmchem International Ltd.; (C) Shelys Pharmaceutical Ltd.; (D) Tanzania Pharmaceutical Industries Ltd.; (E) authentic PZQ. Batch numbers given in parentheses. The aromatic peaks from methyl-4-hydroxybenzoate are highlighted by the * symbol.

Examples of the ^{1}H NMR spectra in each cluster are shown in Fig. 4 and it is noticeable that only the Shelys product generated resonances at δ 6.95 (doublet), and δ 7.95 (doublet), which indicated the presence of a compound having a para-disubstituted aromatic ring structure. This molecule was predominantly responsible for the deviation of the Shelys product from the rest of the samples in the second PC.

3.3. Identification of the para-disubstituted aromatic compound in the Shelys formulation

Examination of the isolated compound by UPLC-MS (in negative ion mode) indicated a molecular weight of 152 whilst examination by ^{1}H NMR spectroscopy indicated possession of a methoxy group. These findings suggested two possible candidates, 4-methoxybenzoic acid and methyl-4-hydroxybenzoate, with the latter identity being confirmed by ^{1}H NMR spectroscopy following addition of standard samples of each compound obtained from Sigma–Aldrich (Fig. 5).

Further analysis confirmed that methyl-4-hydroxybenzoate also accounted for the unusual extra component seen in the

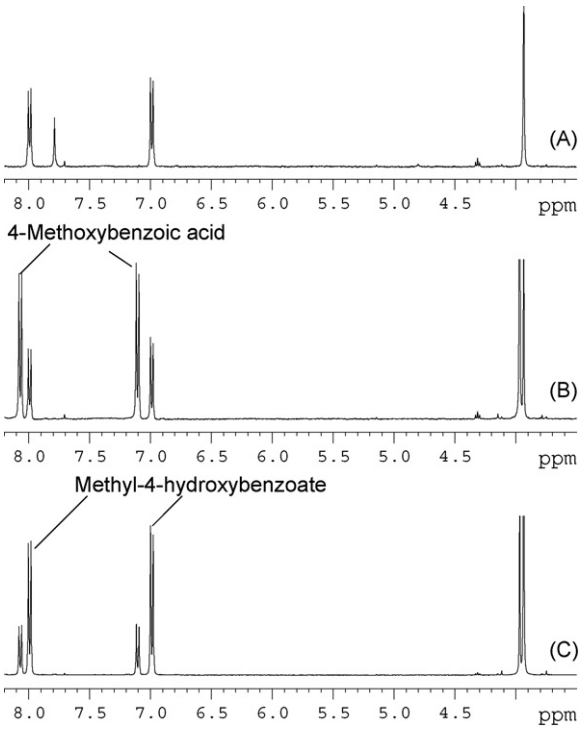

Fig. 5. ^{1}H NMR spectra of para-disubstituted aromatic compound isolated from Shelys PZQ tablet formulation before (A) and after consecutive additions of 4-methoxybenzoic acid (B) and methyl-4-hydroxybenzoate (C).

HPLC–UV chromatogram of the Shelys product. Methyl-4-hydroxybenzoate (methyl paraben) is a known preservative. Therefore, we would assume that it has been deliberately included in the Shelys formulation.

4. Conclusions

Compositional profiling of PZQ tablets by NMR spectroscopy may prove useful in comparing products from different manufacturers or in assessing batch-to-batch variation from a single manufacturer. Additionally, such comparisons could be facilitated by the use of pattern recognition methods such as PCA. In the present study, such analysis revealed significant compositional differences in batches of PZQ tablets from four different manufacturers and, in particular, one formulation was found to contain a preservative, methyl-4-hydroxybenzoate, that was not detected in the other formulations.

Each of the tested batches appeared adequate in regard to the amount of PZQ per tablet as determined by HPLC. However, it should be recognised that the HPLC method for PZQ drug material described in the 2005 edition of the European Pharmacopoeia is not directly transferable to the assessment of PZQ tablets since such tablets may contain additional components that could confuse the analysis. Thus, other tablet components might be wrongly identified as impurities or even mistaken for PZQ.

The approach described here could be readily extended for quality control of other pharmaceuticals, and for detecting counterfeit or substandard anti-infective drugs [22] and has already been applied to assess batch-to-batch variation in traditional herbal remedies (chamomile, feverfew and artemisinin derivatives) [15–17], drugs (ecstasy) [23] and polysorbates [24]. While the proposed analytical strategy is currently out of reach for the majority of settings where PZQ is needed most – due to its high-technology approach, expensive equipment and required expertise – this strategy could become an integral part within sentinel surveillance sites for monitoring the quality of PZQ and other drugs used for the treatment and control of parasitic diseases that are pervasive in the developing world.

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References

[1] WHO, WHO Techn. Rep. Ser. No. 912, 2002.
[2] A. Fenwick, J. Keiser, J. Utzinger, Drug Future 31 (2006) 413–425.
[3] J. Utzinger, J. Keiser, Expert Opin. Pharmacol. 5 (2004) 263–285.
[4] A. Fenwick, L. Savioli, D. Engels, N.R. Bergquist, M.H. Todd, Trends Parasitol. 19 (2003) 509–515.
[5] WHO, WHO Techn. Rep. Ser. No. 849, 1995.
[6] J. Keiser, J. Utzinger, Expert Opin. Pharmacol. 5 (2004) 1711–1726.
[7] M.G. Chen, Acta Trop. 96 (2005) 168–176.
[8] P.J. Lammie, A. Fenwick, J. Utzinger, Trends Parasitol. 22 (2006) 313–321.
[9] J. Kusel, P. Hagan, Parasitol. Today 15 (1999) 352–354.
[10] D. Cioli, Parasitol. Today 14 (1998) 418–422.
[11] E. Renganathan, D. Cioli, Parasitol. Today 14 (1998) 390–391.
[12] C.C. Appleton, A. Mbaye, Trends Parasitol. 17 (2001) 356–357.
[13] S.M. Sulaiman, M. Traore, D. Engels, P. Hagan, D. Cioli, Lancet 358 (2001) 666–667.
[14] European Pharmacopoeia, 5th ed., Council of Europe, Strasbourg, 2005.
[15] Y.L. Wang, H.R. Tang, J.K. Nicholson, P.J. Hylands, J. Sampson, I. Whitcombe, C.G. Stewart, S. Caiger, I. Oru, E. Holmes, Planta Med. 70 (2004) 250–255.
[16] N.J.C. Bailey, J. Sampson, P.J. Hylands, J.K. Nicholson, E. Holmes, Planta Med. 68 (2002) 734–738.
[17] N.J.C. Bailey, Y.L. Wang, J. Sampson, W. Davis, I. Whitcombe, P.J. Hylands, S.L. Croft, E. Holmes, J. Pharm. Biomed. Anal. 35 (2004) 117–126.
[18] W. Winter, R. Deubner, U. Holzgrabe, J. Pharm. Biomed. Anal. 38 (2005) 833–839.
[19] A. Lommen, R. Schilt, J. Weseman, A.H. Roos, J.W. van Velde, M.W.F. Nielen, J. Pharm. Biomed. Anal. 28 (2002) 87–96.
[20] J.K. Nicholson, P.J.D. Foxall, M. Spraul, R.D. Farrant, J.C. Lindon, Anal. Chem. 67 (1995) 793–811.
[21] S. Wold, K. Esbensen, P. Geladi, Chemometr. Intell. Lab. 2 (1987) 37–52.
[22] P.N. Newton, M.D. Green, F.M. Fernandez, N.P.J. Day, N.J. White, Lancet Infect. Dis. 6 (2006) 602–613.
[23] J.F. Carter, E.L. Titterton, M. Murray, R. Sleeman, Analyst 127 (2002) 830–833.
[24] H.V. Dang, A.I. Gray, D. Watson, C.D. Bates, P. Scholes, G.M. Eccleston, J. Pharm. Biomed. Anal. 40 (2006) 1155–1165.