Poster Session 2 – Analytical Chemistry

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Quick detection of glycyrrhizin by an immunochromatographic assay

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Licorice (*Glycyrrhiza* spp.) is one of the most important drugs used as an expectorant, a demulcent and a taste-modifying agent (Conn et al 1968). These pharmacological properties are due to the principal ingredient of licorice, glycyrrhizin, which has anti-viral activity, an anti-inflammatory effect, an antimicrobial effect and hepatoprotective activity (Sasaki et al 2002). To screen glycyrrhizin in plant samples or a biological fluid, a rapid and simple assay system is required for analysis of large numbers of samples and small quantities of test material. Immunoassays using monoclonal antibodies (MAbs) are highly specific methods, therefore they are useful for quantitative and qualitative analysis. We previously reported the preparation of MAb against glycyrrhizin and developed the assay method for glycyrrhizin using anti-glycyrrhizin MAb (Tanaka & Shoyama 1998). An immunochromatographic assay is based on a competitive immunoassay that utilizes antigen–antibody binding properties and provides a rapid and sensitive detection of analyte. We report here the development of a rapid immunochromatographic assay for the detection of glycyrrhizin using anti-glycyrrhizin MAb. An immunochromatographic strip test was developed to detect glycyrrhizin using anti-glycyrrhizin MAb. This qualitative assay is based on a competitive immunoassay in which the detector reagent consists of colloidal gold particles coated with anti-glycyrrhizin MAb. The capture reagents in the assay are glycyrrhizin–human serum albumin (HSA) conjugate immobilized on a nitrocellulose membrane on the test strip. The sample containing glycyrrhizin and the detector reagent pass over the zone where the capture reagent has been immobilized. The glycyrrhizin in the sampl competes for binding to the limited amount of MAb in the detector reagent with the immobilized glycyrrhizin-HSA on the membrane. Therefore, positive samples show no colour in the capture spot zone. The detection limit for the strip test is 250 ng mL^{-1}. The appropriate sample volume size was 200 μL, and the assay can be performed in about 10 min. Glycyrrhizin was detected by the immunochromatographic strip test when various Glycyrrhiza species and food samples were analysed. These results were confirmed by competitive ELISA using anti-glycyrrhizin MAb. Immunochromatographic assay accelerated the analytical procedure and did not require handling reagents. Furthermore, the assay can be available when we need to analyse in the field study. Therefore, the immunochromatographic strip assay was suitable as a rapid and simple procedure for screening glycyrrhizin concentrations in plants, biological fluid and food samples. In conclusion, both immunochromatographic assay described in this report and ELISA were useful methods for the qualitative and quantitative analysis of glycyrrhizin.

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Transfer between different instruments of a near-infrared spectroscopic method for the identification of pharmaceutical excipients

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Near-infrared (NIR) spectroscopy provides an easy, non-invasive technique for the rapid identification of pharmaceutical excipients and has been successfully used within the pharmaceutical industry for many years (Blanco & Romero 2001). Unfortunately, spectral libraries are expensive and time consuming to set up and not easily transferred between different NIR instruments so hindering wider use of the technique. Problems of transfer arise from differences of wavelength accuracy, detector linearity, bandpass effects, stray light and reflectance standards used (Yoon et al 1999). In this study some of the problems of library transfer have been investigated. A library consisting of 108 different excipients' spectra was constructed, 98 had single spectra and ten of the most common excipients (croscarmellose sodium, dibasic calcium phosphate dihydrate, hydroxypropyl cellulose, lactose monohydrate, magnesium stearate, mannitol, microcrystalline cellulose PH101, microcrystalline cellulose PH102, povidone and sodium starch glycolate); each had the mean spectrum of four samples. A fifth sample of each of the ten excipients was also measured and used for validation. Reflectance NIR spectra were measured over the range 1100–2500 nm on two FOSS NIRSystem 6500 instruments. Instrument A was fitted with a Rapid Content Analyser, while instrument B was fitted with a Direct Content Analyser. All spectra were pre-treated by a second-derivative transformation before identification/comparison using Correlation in Wavelength Space (r). Using the spectra measured on instrument A as the reference library, the samples measured on instrument B were all correctly identified apart from the different grades of microcrystalline cellulose. In all cases the r values were greater than 0.982. However, correlation values for the validation samples on instrument B tended to be lower than those on instrument A (e.g., sodium starch glycolate (SSG) and croscarmellose sodium (CS) shown in Table 1). Furthermore, the batch-to-batch variation for each sample was very close to the next best correlated excipient within the library. All five samples of SSG gave a batch-to-batch variation of 1.000–0.986 and all five samples CS gave a variation of 1.000–0.998. The spectral region which displayed the biggest difference between instruments was 1490–1580 nm and corresponded to Wood's peak. Removing this spectral region improved the correlation values (Table 1). Thus, although the spectral library could be transferred for identification, further correction methods would be necessary for qualification.

Table 1 Correlation values for validation samples

Instrument		SSG		CS	
		r	Next nearest match	r	Next nearest match
Full region	A	0.9978	0.9335	0.9993	0.9493
	B	0.9904	0.9217	0.9894	0.9438
Reduced region	A	0.9978	0.9337	0.9993	0.9495
	B	0.9964	0.9304	0.9974	0.9491

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Development of a flow through dissolution method for the determination of metformin hydrochloride and comparison of an in-house versus commercial controlled release formulation

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Our purpose was to develop and validate a flow through dissolution method using a 100 micron path length flow cell for determination of metformin hydrochloride, which possesses a strong UV chromophore. This method was used to establish an in-vitro in-vivo correlation of three metformin hydrochloride controlled release formulations using GastroPlus for comparison. Two commercial formulations and one formulation developed in-house were then compared with the aim of achieving similarity of the in-house formulation when compared to the commercial formulations. This method utilised an automated on-line flow through dissolution system comprising of a Distek Dissolution System 2100C (Distek Inc), Hewlett Packard 8453 UV Spectrophotometer (Agilent Technologies), ICALIS PCP Peristaltic Pump (ICALIS Data Systems), IDIS data collection software (Version 1.25.58, 1996). Dissolution apparatus type was USP 2 paddle method at 50 rev min^{-1} using 900 mL of degassed 0.05 M 0.68% potassium dihydrogen orthophosphate buffer pH 6.8 at 37 ± 0.5°C. Samples were filtered through in line Gelman, 25 mm 1 um glassfibre acrodisc filters (Pall Gelman Laboratory) fitted prior to the 100 micron path length quartz flow cells. Dissolution data was collected every 10 min over an 18-h period. Gastroplus was used to interpret data and predict in-vitro in-vivo correlations followed by f_2 similarity factor analysis. Across a working concentration range of 18–154%, the method developed was linear with a correlation coefficient of 0.9996. Recoveries of metformin HCl from media were acceptable, in the range 96.9–100.9% of label claim. Stability

in solution was acceptable, in the range 99–102% of nominal after 24 h and 99–101% of nominal after 48 h at 37°C. Correlation coefficients for precision were all < 5%. Filter validation confirmed no adsorption of drug to the filter. In-vitro in-vivo correlation was demonstrated and an f_2 similarity factor of 50 was achieved with the in-house formulation when compared with the commercial control. In conclusion, a sensitive, precise and accurate flow through dissolution method was developed, which alleviated the requirement for dilution of samples prior to analysis. Data demonstrates that there is an in-vitro in-vivo correlation. The three controlled release formulations were compared and the in-house formulation has successfully been confirmed as having a statistically similar profile to the commercial formulations.

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Drug testing in rugby union: the views of the players

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Rugby union players are subject to drug testing in accordance with the rules of the IRB (International Rugby Board) (IRB 2003). Analytical chemistry techniques are used in the accredited drug testing programmes (Verroken & Mottram 2003). In 2002 a rugby union player tested positive for ephedrine, a substance banned by the IRB (BBCSport 2002). No action was taken as it was reported that he had taken ephedrine to counteract the effects of influenza symptoms and that he had stated that he was unaware that the preparation contained a banned substance. Although much has been reported in the lay and scientific press on drugs testing and the analytical techniques involved, little has been published on athletes' perceptions of drug testing in sport, including rugby union. Therefore, this paper describes an investigation of the views and experiences of a sample of male rugby union players at different playing levels. Questionnaires were distributed to 238 male rugby union players and 225 were returned (95% response), 50 were professionals, 44 were semi-professional players and 130 were amateurs. One player did not state playing level. Players were asked to indicate their level of agreement or otherwise with the statement "There is very little chance of getting caught using a banned substance at your playing level." The results are shown in Table 1. Respondents were also asked if they were aware if certain named substances were banned by the IRB and the responses are shown in Table 2. The table shows that awareness of anabolic steroids being banned is similar at all three laying levels. However, awareness of other agents, some of which can be bought over the counter without a prescription, tends to be greatest in professionals and least in amateur players, as one might expect. The findings of this study identify there is a lack of knowledge in rugby union players in this sample. The existence of significant numbers of players who are unaware that certain products are banned (Table 2) is of concern. Further, nearly one-third of professionals, two-thirds of semi-professionals and over three-quarters of amateurs believe there is little chance in their being caught if they were to take a banned substance, knowingly or otherwise. The authors recommend that much greater education of rugby union players and perhaps other sports people is required. When considering the findings of this study it would appear that if drugs testing in rugby union were to be significantly increased then, due to ignorance, it is possible that the number of positive tests identified by analytical techniques may be enlightening.

Table 1 Percentage of respondents indicating views on getting caught

	Professional	Semi-professional	Amateur
Strongly agree	8	34	49
Agree	22	34	28
Don't know	8	11	11
Disagree	26	11	9
Strongly disagree	30	7	2
Other	6	2	2

Table 2 Percentage of respondents correctly identifying banned substances

	Professional	Semi-professional	Amateur
Pseudoephedrine	59	40	24
Ephedrine	86	72	50
Anabolic steroids	90	93	90
Clenbuterol	67	59	32
Salbutamol inhaler for non-asthmatic	35	42	21

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The measurement of the degree of substitution in hydroxypropyl-β-cyclodextrin by near-infrared reflectance spectroscopy

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Hydoxypropyl-β-cyclodextrin (HPBCD) has found wide application within the pharmaceutical industry because of its ability to solubilize poorly soluble drug compounds. Its excellent solubility and low toxicity make it especially useful in the formulation of parenteral products. The degree of substitution (DS) has a great impact on the formation and stability of inclusion complexes, and consequently is required to be known. DS values are currently determined by NMR spectroscopy. In this work Near-infrared (NIR) reflectance spectroscopy has been used to determine the DS value in commercial samples. Samples of HPBCD were obtained from Cerestar Inc, with DS values in the range 4.1–6.9. Spectra were measured on a FOSS NIRSystems (Silver Springs, USA) model 6500 near-infrared spectrometer, fitted with a Rapid Content Analyser, over the wavelength range 1100–2500 nm. For each sample, three spectra were recorded and the mean spectrum used for data analysis. All spectra were measured with respect to an 80% nominal reflectance standard (FOSS, part number RSS10088). Mean absorbance spectra were exported to the Unscrambler software package, version 7.6 (Camo, Trondheim, Norway). Calibration models were constructed using partial least square regression (PLSR). Sixteen samples were assigned to a calibration set and eight samples to a validation set such that each set was representative with respect to DS values. Various data pre-treatments were used: multiplicative scatter correction (MSC) to remove particle size effects, and derivatives to remove baseline offsets. First derivative (D1_*n*) or second derivative (D2_*n*) spectra were calculated using an *n* point Savitsky-Golay filter, with a 2nd order polynomial. The results for the PLSR models (all using four factors) are shown in Table 1. The best model (data pre-treatment: MSC plus first derivative, using 21 data points) gave for the calibration set a multiple correlation coefficient (MCC) of 0.986 and root mean standard error of calibration (RMSEC) of 0.14. For the validation set the root mean standard error of prediction (RMSEP) was 0.13 representing an assay error of typically 2–3% on the DS value. These results compare favourably with NMR spectroscopy, and this would allow its replacement in the quality control laboratory by the much faster and simpler NIR methodology.

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Table 1 Partial Least Squares Models

Model	MCC	RMSEC	RMSEP
Abs	0.971	0.20	0.24
MSC	0.988	0.13	0.16
MSC/D1_11	0.977	0.18	0.16
MSC/D1_21	0.986	0.14	0.13
MSC/D2_11	0.980	0.17	0.18
MSC/D2_21	0.978	0.17	0.18

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High-resolution ultrasonic spectroscopy for real time analysis of chemical reactions

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High-Resolution Ultrasonic Spectroscopy (HR-US) is a new technique for material analysis based on the precision measurements of the parameters of

high-frequency sound waves propagating through samples. Sound waves of compressions and decompressions propagate through most materials, including opaque samples, and allow direct probing of micro-structural organisation and intermolecular forces. HR-US high-resolution ultrasonic spectrometers measure two parameters, ultrasonic attenuation and velocity. Ultrasonic attenuation is determined by the scattering of ultrasonic waves in non-homogenous samples (protein aggregates, micelles, liposomes, emulsions, etc.) and fast chemical relaxation in homogenous mixtures. Intermolecular forces are the major contributor to the ultrasonic velocity. Both parameters are physically independent, allowing analysis of different levels of organization of the samples, from primary chemical structure and hydration of atomic groups to supramolecular arrangements, protein self assemblies, gels, emulsions, suspensions, etc. The HR-US family of high-resolution ultrasonic spectrometers provide a universal capability for the monitoring of chemical reactions in real time including: direct detection of chemical reactions by monitoring the change in concentration of the substrates and products, structural effects in polymers and aggregates, changes in molecular weight, etc. This technology is extremely sensitive, non-destructive, requires no markers and can be used in non-transparent samples, such as emulsions, concentrated dispersions and in semisolid materials. The measurements can be performed at low and high concentrations and volumes of 0.03 mL and higher in a broad temperature range with no practical limits on the solvents and components of the reactions. This publication describes the application of high-resolution ultrasonic spectroscopy for direct real time monitoring of crystallisation of lysozyme, formation of micro-emulsions and the hydrolysis of maltodextrin by α-amylase.

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Evaluation of fibre optic technologies for 'in-situ' intrinsic dissolution rate assessment

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This work reports on the progress made in assessing the feasibility of applying in-situ fiber optics to assess the intrinsic dissolution rate (IDR) of active pharmaceutical ingredients (APIs). An evaluation of a commercially available fiber optic instrument against the conventional off-line HPLC analysis is included. In the first part of this study, the suitability of in-situ fiber optic IDR application was measured against eight key parameters — cost (capital, consumables), robustness, resource burden, COSHH, analytical information (speed, compliance), support, software, data analysis and reporting. In the second part of this study, experimental investigations were carried out using a Distek dissolution apparatus fitted with a Wood's intrinsic dissolution die. The dissolution volume used was 500 mL and the rotational speed 100 rev min^{-1}. The dissolution data was captured using fiber optics. Manual sampling from each dissolution vessel was also carried out to enable HPLC analysis. The release levels (in mg cm^{-2}) calculated for this API for both HPLC and fiber optics were plotted against time. The IDR values were determined via linear regression. The results shown in Table 1 indicate that in-situ fiber optic dissolution monitoring gives a similar IDR result to off-line HPLC analysis. Assessment of the key parameters showed that analysis using a fiber optic system offered significant advantages over off-line HPLC. The key differentiating parameters between in-situ fiber optic and off-line HPLC approaches were resource burden, COSHH, speed, data analysis and reporting. Greater efficiency for IDR determination is achievable using in-situ fiber optics than by conventional HPLC enabling considerable time and cost savings. As a result it was decided that fiber optics would be routinely applied for intrinsic dissolution rate assessment where the API possesses an appropriate UV response. Fiber optics can be used to increase the efficiency for measuring the IDR of APIs. The use of in-situ fiber optic technologies to assess IDR can aid in reaching a better understanding of the potential correlation with BCS for pharmaceuticals (Yu et al 2004).

Table 1 IDR data for dissolution of an API at pH 4.5 (n = 2)

Technique	IDR (mg cm^{-2} min^{-1})	
	Mean	s.d.
Off-line HPLC	11.45	0.40
In-situ fiber optics	11.00	0.02

Yu, L. X., et al. (2004) *Int. J. Pharm.* **270**: 221–227