Cuendet, M. et al (1997) *Helv. Chim. Acta* **80**: 1144–1151
Eloff, J. N. (1998) *J. Ethnopharmacol.* **60**: 1–8
Hollinworth, H. (1997) *Professional Nurse Study* **12** (Suppl.): 8–11

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Enhancement of colchicine production and recovery from *Gloriosa superba* root tissue culture in liquid medium by using solid phase extraction

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Plant cell and tissue cultures are increasingly being seen as a new source of existing and novel pharmaceuticals (Tom et al 1991). Colchicine is a secondary metabolite of *Gloriosa superba*, which has anti-mitotic and anti-inflammatory properties and has been used for centuries in the treatment of gout and, more recently, for familial Mediterranean fever (Levy et al 1991), and has been recognized for some time as an anti-tumour agent (Davis & Klein 1980). Root tissues grow rapidly in a liquid medium, with doubling times comparable with suspension cell culture or other organ tissue. Root tissue of *Gloriosa superba* established from callus culture initiated from shoot tissue germinated from seeds. The in situ extraction of plant products from tissue culture can dramatically increase the total amounts of secondary metabolites formed in a typical batch culture cycle or in a continuous bioreactor culture. A significant fraction of colchicine produced by *Gloriosa superba* was observed to be released to the liquid medium. To optimize production and stabilise colchicine, we have developed a strategy for continuous in situ extraction and recovery of colchicine from *Gloriosa superba* root cultures. A solid phase extraction using non-ionic exchange resins, various XAD Amberlite resins, were evaluated as extraction phases for accumulating colchicine. (XAD-4) and (XAD-16) have been investigated and have shown a high adsorption capacity and binding affinity towards colchicine. Amberlite resins (XAD-4) and (XAD-16) were enclosed individually in a bag of nylon mesh and incubated with the root culture in liquid medium for four weeks. At the end of the batch culture the mesh bag was removed and extracted with methanol. The tissues were harvested and extracted, and the amount of colchicine in the liquid medium, tissue and the beads was determined by ELISA assay. The concentration of colchicine in the medium was considerably reduced to less than 10% in the culture medium containing Amberlite resins bags compared with the culture without Amberlite resins bags. Average total colchicine accumulation was increased to 0.98 ($\pm$ 0.12) and 0.85 ($\pm$ 0.13) $\mu g\,g^{-1}$ fresh weight with (XAD-4) and (XAD-16), respectively, compared with the control which was 0.33 ($\pm$ 0.04) $\mu g\,g^{-1}$ fresh weights (n = 3). The average colchicine released to the liquid medium (Medium + Resins) was significantly increased by 3.7 and 3.5 fold with (XAD-4) and (XAD-16), respectively, over unextracted culture medium. Also, Amberlite resins (XAD-4) and (XAD-16) sequestered 90.2% and 92.6%, respectively, of total colchicine accumulated in the culture. Amberlite resins greatly enhanced the total colchicine production at the end of the batch culture by 3.1 and 2.9 fold with (XAD-4) and (XAD-16), respectively. This experiment illustrated that further improvements in colchicine production and recovery were possible by using a continuous in situ solid phase extraction system.

Davis P. J., Klein A. E. (1980) *J. Chromatogr.* **188**: 280–284
Levy, M. (1991) *Pharmacotherapy* **11**: 196–211
Tom, R. (1991) *J. Biotechnol.* **21**: 21–42

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A novel extraction method for the active constituents of feverfew (*Tanacetum parthenium* L. Schultz Bip.)

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Herbal and other natural products represent an area of great growth among alternative medical practices. One of the most commonly purchased herbal medicinal products is feverfew, which is used in the treatment of a wide range of disorders, but perhaps more notably for its beneficial effects in the prophylaxis of migraine and treatment of inflammatory disorders, such as arthritis and rheumatism. The chemistry of the plant is very complex and poorly understood; however, the main constituents of feverfew are generally considered to be sesquiterpene lactones, namely parthenolide, and more recently flavonoids, namely santin (Williams et al 1999). To date, these two classes of compounds have been treated quite separately, although both are considered to contribute to the biological activity, and as such, there exists the possibility of synergistic action. A great wealth of literature exists for the isolation and characterisation of the sesquiterpene lactone content; however, similar information for the flavonoids is very sparse. The current lack of evidence, particularly the absence of a comprehensive knowledge of these active constituents together, formed the basis of this research. The research was directed towards developing a novel procedure to extract the active constituents of feverfew simultaneously, using simple solvent extraction, before isolation and characterisation by TLC and UV spectroscopy, utilising existing literature methods (Greenham et al 2003). Several extraction solvents were investigated and it was found that an acetone–ethanol–water (5:3:2; 10 mL) mixture not only extracted the greatest number of constituents (when compared with existing methods) from 1 g of dried, powdered *T. parthenium* leaf material, but more importantly extracted parthenolide and santin simultaneously. The active constituents were isolated using TLC in a toluene–acetic acid (4:1) solvent system and characterised by their R_f values when compared with reference data, (Greenham et al 2003). Pure parthenolide was available for comparative purposes. Further characterisation involved measurement of λ_{max} values from the methanolic UV spectrum; confirmed using reference data, (Greenham et al 2003). Parthenolide (R_f 0.45; λ_{max} MeOH 210 nm) and santin (R_f 0.58; λ_{max} MeOH 273 nm) were easily extracted and characterised, along with other major sesquiterpene lactone and flavonoid constituents of feverfew. The active components of feverfew are still the subject of much debate. Literature reports have found two main families of compounds associated with activity in bioassays and for the purposes of this research; these have been studied simultaneously using the novel extraction method developed. It is important that future standardisation work involving feverfew continues in this manner; currently, the identity and quality of most feverfew products only depend on the presence and concentration of parthenolide, wrongly ignoring the flavonoid content. An important prerequisite for establishing quality standards is the extraction, isolation and identification of all active components; the novel method developed here could prove a useful tool in minimising the current problems experienced.

Greenham, J. et al (2003) *Phytochem. Anal.* **14**: 100–118
Williams, C. A. et al (1999) *Phytochemistry* **51**: 417–423

Short Talks on Pharmaceutical Analysis

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Use of orthogonal RP-HPLC and LC-MS in the identification, qualification and control of an ethyl carbamate impurity in a new drug substance

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Effective monitoring of synthetic impurities is imperative to ensure that they are eliminated or reduced to acceptable levels in drug substances. The International Conference on Harmonisation (ICH) guideline Q3A(R) "Impurities in New Drug Substances" suggests that new organic impurities should be controlled to a level not more than a qualification threshold (0.15%) for identified impurities, while unidentified impurities should be controlled to a level not more than the identification threshold (0.10%) at the time of registration. The impurity control strategy for a new drug substance is developed concurrently with analytical methods during the early phases of clinical development, adding complexity. Therefore, orthogonal analytical methods and sensitive identification techniques, such as LC-MS, are employed to examine impurity profiles and evaluate mass balance, as well as support impurity identification and qualification. This paper will demonstrate how a multidisciplinary approach is used to overcome this essential problem in pharmaceutical analysis. The routine batch release method for the new drug substance employs a C18 column with 20 mM potassium phosphate buffer pH8/acetonitrile gradient elution and UV detection at 210 nm. This method has been demonstrated to be specific and stability indicating, with suitable analyte stability, precision and linearity for its intended purpose. During development studies to scale up the synthetic process, a recurring impurity peak was observed at elevated levels. Interrogation of the peak of interest using electrospray LC-MS revealed the presence of two co-eluting impurities: an ethyl carbamate homologue of the parent drug, along with a high molecular weight adduct. These impurity structures were screened using a knowledge-based expert system for qualitative prediction of toxicology, DEREK (Deductive Estimation of Risk from Existing Knowledge), which indicated the ethyl carbamate moiety in the first impurity as the only potential toxophore. An orthogonal method, employing a C18 column with a 0.2% perchloric acid/

acetonitrile gradient with UV detection at 215 nm, improved the selectivity between the two impurities. The orthogonal LC method allowed definitive structural assignment and accurate quantification of the ethyl carbamate impurity. This impurity was demonstrated to be present in batches used in pre-clinical toxicology studies without concern. In addition to establishing the qualification of this impurity, identification also enabled postulation of the impurity's origin and its proposed control at an earlier stage of the synthesis.

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Stability of drug combinations commonly used in palliative care

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Admixtures of multiple drugs are frequently used in palliative care of the terminally ill to provide pain relief and to manage other symptoms. Syringe driver devices at the bedside are widely used to administer combinations of analgesics, sedatives and antiemetics by subcutaneous infusion over periods of up to 24 h. Compatibility of such drug mixtures is often assessed visually (check for colour change or precipitation) and by determining that the desired clinical effect is achieved (Twycross et al 1998). However, very little chemical stability data exists for combinations of three drugs. This study was devised to assess the stability of two commonly used mixtures. High performance liquid chromatography (HPLC) was chosen as the method of analysis because it is capable of detecting degradation products as well as any loss of active drug substance. Three rapid, precise and stability-indicating HPLC methods have been developed for the quantitative analysis of diamorphine, midazolam, metoclopramide and levomepromazine. The methods use C18 Kromasil columns and mobile phases containing either acetonitrile/ammonium acetate/diethylamine or acetonitrile/sodium acetate/TMAH. The following combinations were evaluated: diamorphine/midazolam/metoclopramide and diamorphine/midazolam/levomepromazine. The study was conducted at two dosage levels (one high, one low), to detect any concentration-dependent instability. Mixtures were made up in polypropylene pump syringes and stored under ambient light and temperature conditions for 24 h. This simulates the in-use conditions at the bedside. The higher dose combinations were also stored at 4°C for 7 days. Samples were taken from the syringes at intervals and analysed using the validated HPLC methods. Drug substances were quantitatively determined and samples examined for the presence of degradation products. Results show that after 24 h at room temperature, both combinations of drugs at both dosages show no loss of active substance and no detectable degradation products. After 7 days at 4°C, both high dose combinations show no degradation of active drug or presence of degradation products. We conclude that the drug mixtures studied are stable in polypropylene syringes on a bedside syringe driver for the 24 h in-use period. These combinations are also stable for 7 days at 4°C – this allows for limited storage of prepared infusions before use.

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Twycross, R. et al (1998) *Palliative care formulary*. Oxford: Radcliffe Medical Press

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Analysis of single lipopolyamine-DNA nanoparticle formation in non-viral gene therapy by fluorescence correlation spectroscopy using PicoGreen

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Non-viral gene therapy (NVGT) has the potential to revolutionize the way difficult-to-cure diseases are treated, by inserting genes coding for missing proteins (e.g. enzymes or signals) into patients' target cells. DNA nanocomplex formation is the first crucial step in NVGT. This enables lengthy polynucleotides to be stored as nanoparticles, which it is feasible to deliver to target cells. DNA nanostructures (50–150 nm in outer diameter) can self-assemble by the interactions of the cationic lipid vectors with the negative charges of DNA phosphate. This DNA compaction protects DNA from degradation and facilitates gene delivery. Small molecule sensors for DNA intercalation (e.g. ethidium bromide (EthBr), PicoGreen (PG)) have been widely used to determine polycation-DNA interactions, by measuring the fluorescence intensity of probes during condensation. As well as these fluorescent techniques, the formation of DNA nanoparticles can be observed in a light scattering (LS) assay, measuring UV apparent absorption at $\lambda > 300$ nm. Fluorescence correlation spectroscopy (FCS) is a new technique where fluctuations in the detected fluorescence from small sensors are also used to study dynamic processes of DNA nanocomplex formation (Kral et al 2002; Adjimatera et al 2005). The advantages of the FCS approach over standard fluorescence techniques are: use of lower dye concentration; use of lower (nM range) DNA concentration; information about possible condensed plasmid sub-populations; the possibility of monitoring the condensation process at the single molecule level. Employing PicoGreen (PG), a high affinity DNA intercalating agent that only fluoresces when intercalated, we have used FCS to provide more insight (e.g. diffusion time and particle number (PN)), than the steady-state fluorimetric method. PG does not change the hydrodynamic properties of DNA and does not influence the lipopolyamine concentrations necessary for condensation. Additionally, PG requires 10-fold lower staining when compared with previously used markers because of the polyamine moiety structural modification, which efficiently forms salt bridges with DNA phosphate anions, and taken together with the DNA intercalation, this is higher affinity from biphasic binding. We have studied the condensation of linear calf thymus DNA (ct DNA, 13 kilobase pairs) and circular plasmid DNA (pEGFP, 4.7 kilobase pairs) using two lipopolyamines, N^4,N^9-dioleoylspermine (Ahmed et al 2005) and N^1-cholesteryl spermine carbamate. N^4,N^9-Dioleoylspermine efficiently condensed both ct DNA and pEGFP into point-like molecules with diffusion coefficient $(D) = 1.8 \times 10^{-12}\,m^2\,s^{-1}$ and $2.3 \times 10^{-12}\,m^2\,s^{-1}$, and particle number $(PN) = 0.7$ and 0.9 (the theoretical PN under this point-like molecular model is 0.6). Cholesteryl spermine carbamate showed poorer DNA condensation efficiency, even at higher N/P (ammonium/phosphate) charge ratios. Ultimately, a better understanding of DNA condensation, using new probes and analytical techniques, will lead to the development of more efficient DNA condensing agents.

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Adjimatera, N. et al (2005) In: Hof, M. et al (eds) *Fluorescence spectroscopy in biology*. Berlin: Springer-Verlag, pp 201–228
Ahmed, O. A. A. et al (2005) *Pharm. Res.* **22**: In press
Kral, T. et al (2002) *Biophys. Chem.* **95**: 135–144

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Raman microscopy investigation of the phase structure and interactions in hydoxypropyl methyl cellulose and ethyl cellulose blends

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Blends of hydroxypropyl methylcellulose (HPMC) and ethyl cellulose (EC) are widely used as pharmaceutical controlled release coatings and carriers (Rowe 1985). A proposed mechanism for the performance of such coatings is drug release through pores in the insoluble EC matrix formed by dissolution of the water-soluble HPMC phase. Consequently, the function and release profile of a given formulation coated in this manner depends critically on the distribution, morphology and degree of mixing of the blend. Previously, optical microscopy has been applied to elucidate the blend morphology; however, this did not provide any direct evidence for the HPMC/EC distribution (Sakellariou & Rowe 1995). In this study, the capability of Raman microscopy to map composition across a blend film is reported. Tapping mode phase imaging atomic force microscopy (AFM) was used to provide high spatial resolution images of the film structure. Films were prepared by dissolving a mixture of 1:1 w/w HPMC/EC in a solution of 50:50 v/v methanol/dichloromethane. A film was prepared by casting from solution and then cross-sectioned to allow the bulk morphology to be accessed. Raman microscopy was performed using a Witec Confocal Raman Microscope (CRM), equipped with a 785 nm laser. AFM images were recorded using a Veeco Nanoscope IIIa instrument operating in Tapping Mode. SEM images were obtained using a LEO 1430 VP microscope. AFM images reveal the presence of circular domains at the sectioned surface. In particular, AFM phase imaging highlights smooth texture inside these domains compared with the granular surroundings. The chemical nature of these domains has been determined using Raman microscopy. By selecting a chemically characteristic wavelength on a spectrum, is it possible to draw a distribution map of the selected species over a scanned area. However, both HPMC and EC are cellulose-type related materials and present very similar Raman spectra. The chemical difference here between each molecule is due to the substituted groups, which present subtle differences in the fingerprint region of the Raman spectrum. The ether C-O-C stretch gives

rise to strong bands at 880 cm^{-1} for EC and 890 cm^{-1} for HPMC; and the difference in the substituents also distinguishes bands at 1264 cm^{-1} for EC and 948 cm^{-1} for HPMC. With careful selection of appropriate bands, chemical mapping by Raman microscopy indicates complete phase separation of the two polymers. EC is present in the blend as domains of size 3–10 μm dispersed in an HPMC matrix. This suggests a reversed phase structure to that often postulated for controlled release mechanism for this sample. Spectral features may also be altered through the formation of hydrogen bonds between the two components which have been previously postulated. Here we show that the two phases do not form hydrogen bonds at their interfaces. In conclusion, Confocal Raman microscopy is a powerful technique as it can be used to chemically distinguish controlled release components. The new results described here open a new door to further investigations of the nature of controlled release films.

Rowe, R. C. (1985) In: Elnence, A. T. (ed.) *Materials used in pharmaceutical formulation, critical reports in applied chemistry*. Oxford: Blackwell Scientific Publications, pp 1–36
Sakellariou, P., Rowe, R. C. (1995) *Int. J. Pharm.* **125**: 289–296

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Temperature dependent terahertz pulsed spectroscopy of carbamazepine tablets

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Terahertz pulsed spectroscopy (TPS) is a novel spectroscopic technique that has shown great potential in characterizing crystalline materials. It has proved to be a very powerful tool to investigate crystalline materials and is used to distinguish between polymorphic forms in drugs (Taday et al 2003; Strachan et al 2004, 2005). In this study, we use TPS to simultaneously monitor changes in the crystalline structure of both drug and excipients in a formulated tablet for the first time. Sample tablets were made by compressing 200 mg carbamazepine (CBZ) polymorph III, 80 mg α-lactose monohydrate (αLMH) and appropriate amounts of talc and magnesium stearate with a 2-ton load for 3 min. The tablets were placed into a brass ring with an aperture of 8 mm and inserted into a heatable transmission cell with no windows. Spectra of the tablets were recorded between 303 and 443 K on heating the sample and cooling it down again at a rate of 2 K min^{-1}. To interpret the results from the tablet heating measurements, temperature-dependent measurements of CBZ form I and III, αLMH, α-lactose anhydrate (αLAH) and β-lactose anhydrate (βLAH) were carried out using the same methodology. In the TPS spectra of the formulated tablet all spectral features of CBZ form III at 28. cm^{-1}, 41.7 cm^{-1} and 61.3 cm^{-1}, as well as the peaks of αLMH at 18.0 cm^{-1} and 46.4 cm^{-1}, could be observed. On heating the tablets up to 443 K, the peaks decreased in intensity and red-shifted. At 443 K the peak of form I emerged at 30.6 cm^{-1}, while the rest of the spectral region exhibited diffuse absorption indicating the melting event. On cooling the samples down to 303 K, all peaks of carbamazepine form I emerged, sharpened up and blue-shifted to their characteristic positions at 31.8 cm^{-1}, 45.7 cm^{-1} and 52.5 cm^{-1}. The experiments of heating and cooling αLAH, βLAH and CBZ form I showed no transformation processes. αLAH exhibits peaks at 36.2 cm^{-1} and 61.7 cm^{-1}, βLAH at 40.3 cm^{-1}, 63.5 cm^{-1} and 72.8 cm^{-1}, while CBZ form I has peaks at 31.8 cm^{-1}, 45.7 cm^{-1} and 52.5 cm^{-1}. All these features showed red-shift, peak broadening and a decrease in intensity on heating and the reverse processes on cooling the pure samples down again. However, for αLMH a change in the spectra was observed. On heating this sample up to 403 K the αLMH peaks red-shifted and broadened, interestingly, on further heating up to 443 K the spectra showed only diffuse absorption rather than any spectral features. As the sample was cooled down again no peaks emerged and the spectra remained unchanged. This indicates that the dehydration of the monohydrate in the compressed pellet is altered and the diffusion of the hydrate water out of the pellet is hindered. Even though further research is necessary to fully understand the dehydration mechanism of αLMH in a compressed pellet, TPS clearly showed the ability to monitor changes of polymorphic form in pharmaceutical tablets in situ.

Strachan, C. J. et al (2004) *Chem. Phys. Lett.* **390**: 20–24
Strachan, C. J. et al (2005) *J. Pharm. Sci.* **94**: 837–846
Taday, P. F. et al (2003) *J. Pharm. Sci.* **92**: 831–838

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New applications of high-resolution ultrasonic spectroscopy for analysis of molecular processes in pharmaceutical research and industry

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High-resolution ultrasonic spectroscopy (HR-US) is a new, non-destructive technique based on precision measurements of parameters of acoustical waves (velocity and attenuation) at high frequencies propagating through materials. Attenuation is determined by the scattering of ultrasonic waves in non-homogenous samples (e.g. suspensions) and fast chemical relaxation in homogenous mixtures. The elasticity of medium is the major factor that determines the ultrasonic velocity. Two independent parameters allow the probing of different levels of sample organization, from chemical structure and hydration of atomic groups to microstructure of gels and dispersion. Unlike other methods, optical transparency is not required as ultrasonic waves propagate through most materials, including opaque samples. The HR-US instruments with small sample volume (down to 0.03 mL) can be used for the analysis of composition, aggregation, gelation, micelle formation, crystallization, sedimentation, enzyme activity, conformational transitions, ligand binding and many other key processes in drug manufacturing. Capable of dealing with a wide range of samples and dynamic processes, the HR-US is a new tool for product quality control in real time. The latest development of the ultrasonic measurements at controlled excessive pressure allows the analysis of liquids with low boiling point (e.g. inhalers) and dissolved and dispersed gases in them. Automatic titration system provides measurement of the phase diagrams in surfactant/co-surfactant systems, molecular binding and other processes. The objective of this presentation is a demonstration of extended capabilities of HR-US for pharmaceutical research and industry as illustrated in several new applications. In the first application, the HR-US measurements were used for monitoring of particles size and their volume fraction upon creaming in a suspension of inhaler drug. Faster kinetics of the creaming is detected at higher initial concentration of the drug, which is attributed to rapid aggregation of drug particles in concentrated suspension. The second application deals with the effect of insertion of a respiratory gas into a perfluorocarbons liquid and its emulsion, used in synthetic blood substitute. The HR-US technique enables the kinetics of solubilization of gas in samples to be followed, as well as determination of the solubility of various (O_2, CO_2, N_2) gases in perfluoracarbons. The third example is detection and analysis of phase diagram of micro-emulsions. This example illustrates how microstructural transitions precluding formation of microemulsion, particle size and state of water in microemulsion region, microstructural characteristics of emulsion and biocontinous phases can be detected from ultrasonic titration profiles. Monitoring of crystal formation, and in particular kinetics of the reaction, is an important part of routine analysis in the pharmaceutical industry. The benefit of HR-US measurements for the process control in the batch crystallization of pharmaceutical compounds is illustrated by the ultrasonic measurements of the structure, size and amount of crystals during the crystallization in solutions. HR-US has enormous potential for analysis of materials, formulations and processes used in pharmaceutical industry. It provides greater productivity and cost saving for end-users, allowing the range of analysis that currently have to be done by several separate blocks of techniques. The capability of High Resolution Ultrasonic Spectroscopy has been demonstrated for characterization of micro-structural and molecular processes in pharmaceutical systems, including analysis of creaming in inhalers, structural transitions in microemulsions, dissolving of respiratory gases in synthetic blood substitutes and crystallization of proteins.

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Comparison of near-infrared spectroscopic methods for the identification of pharmaceutical excipients and active drug ingredients

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Identification of excipients is commonly accomplished by invasive chemical methods. Near-infrared (NIR) spectroscopy is an ideal, versatile and rapid alternative that can be carried out in the warehouse or holding location of a

manufacturing site so reducing the quarantine time. A key paper from Blanco & Romero (2001) proposed the use of cascading libraries for NIR spectroscopy. In this work, an NIR spectral library of excipients and active pharmaceutical ingredients (APIs) was constructed and used to compare five identification methods. Reflectance NIR spectra were collected over the range 1100–2500 nm on a FOSS 6500 Spectrophotometer fitted with a Rapid Content Analyser for 210 pharmaceutical compounds. Five pattern recognition methods were compared, based on second derivative spectra: Wavelength Correlation (WC); WC with wavelength selection (WCWS); Maximum Distance in Wavelength Space (MDWS); Peak Positioning (PP); and Soft Independent Modelling of Class Analogies (SIMCA). A reduced library of 55 compounds (containing at least eight batches of each substance) was used to optimise each method. The number of compounds correctly identified by each individual method is given in Table 1. SIMCA was the best method, but it required extensive data analysis for little advantage compared with the other methods. The next best method was PP; but it was discarded because robustness was an issue over time. MDWS came next, but multiple sample spectra are required for this procedure. While WC was the worst performing method, it was easy to update (i.e. add new compounds) and it also gave a good indication of the 'problem' compounds highlighted by the other methods. Identification was best performed by using a cascading approach, initially using WC to divide the spectral library into groups. Each group was then sub-divided sequentially using WCWS, MDWS and SIMCA. Where the group contained both chemically and physically different compounds, then WCWS was next applied. For groups containing only physically different compounds (e.g. particle size), or mixtures with different ratios of components, then MDWS was optimal. SIMCA was finally used for any groups that still remained unresolved. The procedure was initially applied to the library of 55 compounds; 36 compounds were correctly identified with the remainder grouped into starch and starch derivatives or compounds that differed only by the grade of material (Table 1). Applying the procedure to the 210 compounds, for which there were 1–91 batches for each compound, 167 compounds were correctly identified, leaving 43 that fell into 14 groups. Of these 14 groups, only one involved different chemical compounds (ceratonia, guar galactomannan and tara gum). In this study, there were insufficient batches for these compounds to allow further discrimination. In conclusion, while SIMCA is the best individual method, the cascading approach advocated is much better.

Table 1 Number of compounds identified using different pattern recognition methods

Procedure	Method				
	WC	WCWS	MDWS	SIMCA	PP
Single method (n = 55)	24	—	29	33	30
Cumulative procedure (n = 55)	24	29	33	36	—
Cumulative procedure (n = 210)	124	137	151	167	—

Blanco, M., Romero, M. A. (2001) *Analyst* **126**: 2212–2217

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Identification of counterfeit Cialis, Levitra and Viagra tablets by near-infrared spectroscopy

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The Busan Regional Korea Food and Drugs Administration (BRKFDA) analysed suspected Cialis, Levitra and Viagra tablets by high-performance liquid chromatography (HPLC), but the analyses took a long time and could not differentiate between excipients. Near-infrared (NIR) spectroscopic analysis was therefore examined as it had previously been used successfully for the identification of counterfeit Viagra tablets (Wilson & Moffat 2004) and to compare parallel imports (Yoon et al 2004). Different authentic batches of Cialis (20 mg tadalafil), Levitra (20 mg vardenafil) and Viagra (100 mg sildenafil) tablets were purchased from a local London pharmacy and authentic Viagra tablets were also obtained from seven different countries. Counterfeit tablets were supplied by the BRKFDA. They were scanned using a FOSS NIRSystems 6500 spectrometer fitted with a Rapid Content Analyser over the range 110–2500 nm taking only 40 s per tablet. Data processing was carried out using Vision software (FOSS) and The Unscrambler (CAMO). Reference analysis (HPLC) was made using a CAPCELL PAK C18 column. All the counterfeit Cialis tablets could be identified from their NIR spectra by using a correlation in wavelength space threshold of 0.90 (using second derivative and standard normal variate (SNV) pre-treatments). A principal components analysis (PCA) model using three principal components easily distinguished counterfeit from authentic Cialis tablets. HPLC analysis revealed that one batch of the counterfeit tablets contained 103 mg sildenafil instead of 20 mg tadalafil. Examination of the NIR spectra of the authentic and counterfeit Levitra tablets showed only small differences. Also, the NIR spectra of the counterfeit Levitra tablets were very similar to authentic Viagra tablets. This was explained by HPLC analysis, which showed that the counterfeit Levitra tablets each contained 1.3 mg sildenafil (the active ingredient of Viagra tablets) and 17 mg of tadalafil (the active ingredient of Cialis tablets) instead of 20 mg of vardenafil. Adequate discrimination was obtained when SNV and second derivative transformations were used together with correlation in wavelength space and maximum wavelength distance algorithms. PCA was the most effective tool for identification. Authentic and counterfeit Levitra tablets could easily be differentiated from the plots by using only the first two principal components. This was true also for centre of gravity plots (Yoon et al 2000). The NIR spectra and second derivative spectra for the authentic and counterfeit Viagra tablets were very similar with correlation in wavelength space not discriminating them. However, maximum wavelength distance (using SNV and second derivative pre-treatments) could discriminate authentic from counterfeit preparations using a threshold of six. Much better was the use of PCA, which could barely differentiate batches of authentic tablets from seven different countries using soft independent modelling class analogy and six principal components, but could easily show the difference between authentic and counterfeit tablets using this model. In conclusion, NIR spectroscopy using PCA is an effective, fast and non-destructive method for identifying counterfeit Cialis, Levitra and Viagra tablets.

Yoon, W. L. et al (2000) In: Davies, A. M. C., Giangiacomo, R., (eds) *Near infrared spectroscopy: proceedings of the 9th International Conference*. Chichester: NIR Publications, pp 547–550

Yoon, W. L. et al (2004) *J. Pharm. Biomed. Anal.* **34**: 933–944

Wilson, N. D., Moffat, A. C. (2004) *J. Pharm. Pharmacol.* **56** (Suppl.): S-3

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Enhanced release of acyclovir from intravaginal rings using common vaginal excipients

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The present scale of the HIV pandemic and the failure to develop an effective vaccine have forced scientists to evaluate alternative preventative strategies for reducing the rate of HIV transmission. Since over 90% of new infections result from heterosexual intercourse, the most viable, short-term strategy being pursued is the development of vaginal HIV microbicides. In particular, vaginal rings providing long-term, controlled-release of antiretrovirals over days/weeks/months are actively being investigated for this purpose. Several studies have demonstrated that prior infection with herpes simplex virus type 2 (HSV-2) is an important risk factor for HIV acquisition (Holmberg et al 1988), attributed to the presence of vaginal lesions and subsequent infiltration of CD4 cells to the lesion sites. Therefore, preventing and treating HSV-2 vaginal infections may be a useful strategy in reducing the incidence of female HIV infection. In this study, we evaluate the potential for controlled release of the anti-HSV-2 drug acyclovir from silicone vaginal rings. Silicone, matrix-type vaginal rings containing 10% w/w acyclovir and optionally 15% w/w of various pharmaceutically-acceptable, hydrophilic, vaginal excipients (crosscarmellose, polyacrylic acid, ascorbic acid, octyl dodocanol, povidone iodine, undecylenic acid) were manufactured according to standard methodologies (Malcolm et al 2003). In vitro release studies (37°C, 100 mL aqueous release medium, orbital incubation) were performed to assess the potential of each excipient for providing enhanced release of acyclovir from the silicone devices. Briefly, samples of the release medium were taken daily over a 14-day period, the release medium replaced to ensure sink conditions, and the samples subsequently analysed by UV-HPLC for determination of drug concentrations. For