the moisture uptake characteristics, whereas acetone was used to detect the presence of amorphous material (Mackin et al 2002). The moisture sorption profiles exhibited a high degree of variability, with some batches sorbing more than 1% w/w moisture above 80% relative humidity. This unusually high moisture uptake for a crystalline compound has been correlated with the presence of variable levels of sodium chloride (100–500 ppm) on the surface of the drug substance particles. The acetone sorption profiles for freshly micronised batches exhibit a sharp decrease in weight after the samples sorb 0.2% w/w acetone. This weight loss is due to the re-crystallisation of small amounts of amorphous material (< 5% w/w) generated during micronisation. To further examine the impact of this amorphous material, the dispersive and polar components of the surface energy were determined using pulse IGC (Surface Measurement Systems Ltd, UK) at infinite dilution. Experiments were performed at 30°C and 0% relative humidity using a helium carrier flow rate of 10 mL min^{-1}. The dispersive surface energy was determined by eluting 3% v/v injections of a homologous series of alkanes (heptane, octane, nonane and decane). The polar component of the surface energy was determined using acetone, ethanol, acetonitrile and ethyl acetate as the elutants. The IGC data for a typical batch of micronised drug substance are shown in Table 1 and indicate that micronisation significantly increases the dispersive surface energy in addition to changing the acidic/basic nature of the highest energy sites. The data also show a significant reduction in the dispersive and polar surface energy over time, which is probably due to the re-crystallisation of the amorphous material on the surface of the particles. The reduction in surface energy occurs over a period of 1–2 years and the rate of change is affected by the presence of sodium chloride on the surface of the particles. To improve the granulation process and reduce the risk of dissolution failures, the granulation processing conditions have been modified and subsequently no further dissolution failures have occurred. Further studies to investigate the relationship between the surface properties of the drug substance and the granulation behaviour of the formulation are ongoing.

Table 1 Change in the dispersive and polar surface energy of micronised drug substance batches

Drug substance age (weeks)	Dispersive surface energy (mJ m^{-2})	Ka	Kb
Unmilled	34.7 (0.8)	0.10 (0.00)	0.09 (0.00)
0	87.3 (0.8)	0.18 (0.01)	0.00 (0.00)
11	78.6 (1.7)	0.16 (0.00)	0.01 (0.01)
21	71.2 (1.0)	0.15 (0.00)	0.00 (0.00)
30	68.6 (3.3)	0.15 (0.00)	0.00 (0.00)

Ka and Kb are dimensionless numbers referring to the acidic and basic nature of the surface, respectively.
The values in brackets represent the standard deviation for the two columns

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Use of functional polymers for treatment of pulmonary cystic fibrosis

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Cystic fibrosis (CF) is a hereditary disorder caused by mutations in the Cystic Fibrosis Transmembrane Regulator (CFTR) coding gene. Prognosis and treatment of CF has improved significantly over the past few decades, increasing the life expectancy of the patients (FitzSimmons 1993), although the development of novel therapeutics is required to overcome the present problem of antimicrobial resistance and currently available expensive and time consuming treatments. The issue with current treatment is mainly the mechanisms of delivery. The highly viscous mucus in the lungs of CF patients somewhat compromises the activity of the drugs. The pathogenic microorganisms infecting the CF patients form a safe microenvironment within the inspissated mucus barrier, protecting them from any antibiotics/drugs being administered. As a consequence of this, the microorganisms are becoming resistant to the available antibiotics. To solve this problem, this project aims to develop a drug delivery system that will overcome the mucus barrier, hence aiding the delivery of the accompanying drug. The utilisation of biodegradable polymers for therapeutic uses has been investigated since the early 1970s. To overcome the lack of chemical functionality and limited range of variable physico-chemical properties of polymers, such as PLGA, biodegradable polyesters with backbone functionality have recently been developed. This allows the attachment of chemical moieties or drugs, as well as controlled encapsulation and release of desired molecules (drugs, peptides, proteins). In addition, the physical characteristics (hydrophilicity/hydrophobicity) of the polymer can easily be manipulated by varying the backbone chemistry. We have prepared a family of novel, functionalised polyesters with different backbone chemistry and of various molecular weights via the enzyme-catalysed transesterification of a combination of activated di-acids, glycerol and lactone monomers (Kobayashi 1999). Lipase region-selectivity for primary hydroxyl groups produces linear polyesters with pendant hydroxyl groups that were used for the attachment of drugs or the modification of the polymer properties. From these polymers, submicron sized colloidal particles were prepared via emulsion-solvent evaporation (Song et al 1997). This process was optimised to achieve the best particle formation. The effect of polymer chemistry on the ability to form discrete particles was analysed using Scanning Electron Microscopy. Addition of enzyme or drug to the aqueous or organic phase enabled encapsulation of these species into the particles. Release profiles of both the chosen model enzyme (chymotrypsin) and the drug (ibuprofen) were obtained by adding the particles to a buffered system and taking timely samples. The chymotrypsin released was estimated spectrophotometrically and its proteolytic activity determined using the azocasein proteolytic assay. In addition, the enzyme's hydrolysing activity toward mucin as substrate was measured on the developed mucinolytic assay. The released ibuprofen from the samples was estimated using HPLC. These particles will be further developed as a novel pulmonary drug delivery system designed to incorporate novel active mucinolytic enzymes to aid the delivery of an appropriate drug through the mucus barrier of patients with cystic fibrosis.

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Short Talks on Pharmacognosy

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Studies on the anti-psoriatic activity of gossypol and its derivatives followed by pre-formulation and formulation studies of gossypol into a topical dosage form

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Gossypol, a natural anti-inflammatory compound, has been studied extensively since the discovery of its in vivo male antifertility activity in the late 1960s and has since shown anti-viral, anti-parasitic and anti-tumour activity. Psoriasis is a multifactorial skin condition characterised by benign keratinocyte hyper-proliferation, skin inflammation, defective keratinisation, altered dermal vasculature and insufficient anti-oxidant activity. In this study the in vitro anti-psoriatic activity of gossypol and its derivatives was evaluated using an anti-proliferative assay and an anti-oxidant assay (Dodou et al 2005). In the anti-proliferative study, the sensitivity of an HPV-16 keratinocyte cell line to each compound was determined using an MTT viability assay. The compounds that showed increased inhibition against keratinocyte proliferation were subsequently tested for their anti-oxidant effect against iron/ascorbate dependent lipid peroxidation, using the thiobarbituric acid (TBA) test. Racemic gossypol ($GI_{50} = 5.4 \pm 0.03\ \mu M$) and its enantiomers were the most potent compounds against the proliferation of HPV-16 keratinocytes, followed by the half-Schiff's bases ($GI_{50} = 15–50\ \mu M$), racemic gossypolone ($GI_{50} = 47.3\ \mu M$) and the bis-Schiff's bases ($GI_{50} > 100\ \mu M$). A comparison was made with the data from the MTT assays on HPV-16 keratinocyte cell lines using methotrexate ($GI_{50} = 148\ \mu M$) and dithranol ($GI_{50} = 0.58\ \mu M$). All tested compounds showed similar anti-oxidant activity ($IC_{50} \approx 17\ \mu M$) and were more potent than the positive control propyl gallate ($IC_{70} = 100\ \mu M$). Pre-formulation and formulation studies were then conducted on racemic gossypol, which was the most active compound according to the biological assays. The pre-formulation studies included saturation solubility in hydrophilic and lipophilic vehicles, compatibility with excipients, partition co-efficient over pH range 2–8, and physicochemical stability in solution under extreme light, heat, acidic, basic

and oxidising conditions (Dodou 2004). Gossypol showed better solubility in lipophilic vehicles ($> 3\,mg\,mL^{-1}$) than hydrophilic ones ($< 1.5\,mg\,mL^{-1}$) and its water solubility was $0.075\,mg\,mL^{-1}$. It was compatible on storage with commonly used excipients in tetrahydrofuran (THF) solution at 25°C for 6 days. It was found to be stable in acidic, basic and high temperature conditions but was prone to oxidative and photolytic degradation. Its logP value was around 5–6 at pH < 5. The oil in water (o/w) cream of racemic gossypol ($0.065 \pm 0.03\%$ w/w) was physically and chemically stable on storage at temperatures below 30°C for 9 days, had pH = 3.2 and a median oil droplet diameter of 20 μm. In vitro release studies using Franz diffusion cells and a regenerated cellulose membrane showed that the cumulative flux of gossypol was $6.3\,\mu g\,cm^{-2}$ after 9 h. Gossypol was shown to be the most potent inhibitor of keratinocyte proliferation in the anti-proliferative MTT assay, a potent antioxidant in the TBA assay, and its formulation into a topical dosage form was feasible. The above findings, in conjunction with its low human toxicity and lack of mutagenic effects, make gossypol a good candidate for the topical treatment of psoriasis.

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Characteristics of traditional Chinese herbal medicine (TCHM) retail outlets in central London: preliminary results of a cross-sectional study

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In the UK, unlicensed traditional Chinese herbal medicines (TCHMs) are widely available for over-the-counter purchase from TCHM retail outlets without the involvement of a statutorily regulated health care professional. Pharmacovigilance (safety monitoring) for herbal medicines is in the early stages of development (Barnes 2003) yet, in recent years, safety concerns have emerged associated with Chinese herbal remedies prepared by TCHM practitioners and manufactured TCHMs available for purchase from TCHM retail outlets (Barnes et al 2004). These issues raise questions about aspects of TCHM outlets, yet there is a lack of formal study of practices of such shops in the UK. This study aimed at exploring the characteristics of TCHM retail outlets in central London, including types of products sold and medical uses/conditions for which TCHM is promoted. A semi-structured questionnaire, which included questions regarding information visible inside and outside TCHM outlets, was designed and developed, tested for face validity and piloted on five TCHM retail outlets outside the chosen study area. Potential TCHM outlets, including complementary medicine providers, health-food stores and pharmacies, in the study area (W1 postcode) were identified systematically by searching the Yellow Pages on-line directory. After a screening procedure (physically visiting every street in W1), 12/173 (7%) outlets were classified as TCHM retail outlets. A letter describing the study was posted to each outlet one week before the data collection period. Data were collected for the 'outside' of all 12 outlets, and detailed 'inside' observations were done for the four outlets consenting to this. Overall, 11/12 outlets displayed manufactured TCHM products, and nine used drawers or transparent jars to display Chinese crude herbs. Eight of the 12 outlets listed medical uses/conditions visible outside the shop; the median number was 25.5 ($Q_L = 16.25$, $Q_U = 59.5$). There were 274 occurrences of 137 different terms for uses/conditions; each term was counted once only for each shop. Similar terms were combined to produce 108 use/condition categories. Table 1 presents the three most frequently listed categories for the three most common therapeutic areas, after classification by BNF chapter (BMA and RPSGB 2005). Other uses/conditions listed of particular interest include cancer, diabetes, HIV infection and contraception. Also, 77 TCHM-related advertisements were identified within 11 shops; of these, 38 were associated with specific uses/conditions, most commonly skin problems, weight loss and hair loss. TCHM retail outlets in central London sell both crude herbs and manufactured TCHM products. These outlets readily display names of serious medical conditions on their premises, visible to passers-by, which at least implies that TCHMs can be used to prevent, treat or cure these conditions.

Table 1 Most frequently use/condition categories listed

BNF chapter	Use/condition category	n (% of total N; N = 274)
Central nervous system	Stress/Anxiety/Relaxation	10 (3.6%)
	Obesity/Sliming/Weight loss	8 (2.9%)
	Insomnia/Sleeplessness	7 (2.6%)
	Total	53 (19.3%)
Obstetrics, gynaecology and urinary-tract disorders	Infertility	8 (2.9%)
	Menstrual problems	7 (2.6%)
	Impotence	6 (2.2%)
	Total	39 (14.2%)
Skin	Hair Loss	7 (2.6%)
	Eczema	6 (2.2%)
	Psoriasis	6 (2.2%)
	Total	37 (13.5%)

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Anti-proliferative extracts from a Welsh sponge

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Marine invertebrates are a rich source of natural products; the wide range of chemical diversity exhibited by secondary metabolites isolated from these organisms is particularly valuable to drug discovery. The anti-proliferative activity of marine natural products is well documented: 22 such compounds are currently undergoing clinical trials for a variety of conditions, 16 of these are under evaluation as new anti-cancer drugs (Newman & Cragg 2004; Simmons et al 2005). Marine Porifera (sponges) have yielded a wide range of interesting natural products. They are usually associated with warm or tropical seas but are also relatively common in colder waters. British sponges, and their medicinal properties, are largely unexplored and have untapped potential for discovery of novel drugs. A preliminary study of the potential for anti-cancer drug discovery in British Porifera has been undertaken. The growth inhibitory properties of a *Hymeniacidon sp*. sponge from southwest Wales has been evaluated in two human cancer lines, MCF7 breast carcinoma and A549 small cell lung cancer, using previously published methodology (Gee et al 2003). The dried, powdered sponge was extracted with solvents of increasing polarity (dichloromethane–ethyl acetate–methanol) yielding three fractions and an insoluble residue. To determine anti-proliferative activity, stock solutions of each fraction in ethanol were further diluted to suitable concentrations and added to the cell culture medium. All experiments were conducted against an ethanol control, growth inhibition is reported as a percentage of this control and no carrier effects were observed. The dichloromethane and methanol extracts displayed anti-proliferative activity in both cell lines. In particular, the dichloromethane extract demonstrated extremely potent activity against the A549 cells, inhibiting the growth of these cells by >99% at $45\,\mu g\,mL^{-1}$. Analysis of this fraction by thin layer chromatography (TLC) revealed it was a mixture of several distinct compounds. Preparative silica TLC employing chloroform (100%) as eluent was used to separate the mixture and a further five fractions were obtained for analysis in the MCF7 and A549 cell lines. The most polar extract from this separation displayed excellent anti-proliferative properties: 98% growth inhibition at $30\,\mu g\,mL^{-1}$ in A549 cells. In the MCF7 cell line, 88% inhibition at $30\,\mu g\,mL^{-1}$ and 20% inhibition at $3\,\mu g\,mL^{-1}$ was observed. Anti-proliferative effects were negligible at lower concentrations in the A549 cells (1% at $3\,\mu g\,mL^{-1}$). Interestingly, two fractions from the preparative TLC separation showed moderate mitogenic properties, one promoted cell growth by 20% (at $50\,\mu g\,mL^{-1}$) in the MCF7 cells and 16% at the same concentration in the A549 cell line. In conclusion, preliminary investigations have identified a number of bioactive extracts from a Welsh sponge, identifying a promising area for drug discovery. Mitogenic and anti-proliferative effects have been observed, but in particular, certain extracts have highly potent growth inhibition properties against human breast and lung cancer cell lines. Further studies are underway to characterise the natural products responsible for these properties and to identify lead compounds for further development.

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Structures of norditerpenoid alkaloids from *Delphinium* Pacific Giant seeds

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Plants in the genera *Delphinium* and *Aconitum* are a major source of norditerpenoid alkaloids. Some of these alkaloids are highly toxic to mammalian species and therefore their mode(s) of action are of interest to biological and medicinal chemists and pharmacologists. Pelletier & Joshi (1987) summarized the early X-ray crystallographic studies of norditerpenoid alkaloids. These norditerpenoid alkaloids have been categorized into three broad groups: the aconitine-type, which lacks an oxygen function at C-7; the lycoctonine-type, characterized by bearing an oxygen function at C-7; and finally three synthetic compounds where X-ray data have been published (Pelletier & Joshi 1987). These natural products, and their derivatives and analogues, are ligands with important biological activity at selected ion channels. The biological activity and aspects of the history and ethnopharmacy of *Delphinium* usage have been elegantly covered by Benn & Jacyno (1983). Ground *Delphinium* Pacific Giant seeds (500 g) were extracted in a soxhlet thimble sequentially with: hexane, dichloromethane and ethanol (5 cycles each, 2 L scale) (Goodson 1943). After concentration in-vacuo, the residues from each extract were extracted with 0.5 M sulfuric acid (4×100 mL). The combined acidic layers were basified with 5 M NaOH; each fraction was back-extracted with dichloromethane (4×150 mL). The combined organic extracts were washed with water (1 ± 50 mL), dried ($MgSO_4$) and concentrated in-vacuo. Total alkaloid yields were: the hexane extract, 4.0 g; the dichloromethane extract, 1.8 g; the ethanol extract, 5.9 g. TLC analysis (cyclohexane–chloroform–diethylamine 5:4:1) showed that MLA and delpheline were present in all three solvent extracts (Goodson 1943). MLA and delpheline were isolated by column chromatography and their structures established on the basis of ^{1}H, ^{13}C, DEPT, COSY, HMQC and HMBC NMR spectroscopic techniques. Furthermore, delpheline was recrystallized from ethanol–hexane (1:1) and studied for the first time by X-ray crystallography. As a result, the conformations of the six rings are: A and E, chair; D, half-chair (boat flattened at C-15); C and F, envelopes; and B, boat. Three other known alkaloids, namely aconitine, mesaconitine and lycoctonine, were also studied by X-ray crystallography for comparison. All showed similar conformations, with only slight differences in the shape of ring D. It is interesting to note that these similar alkaloids differ considerably in their biological activity, which seems to depend more upon the patterns and types of substitution than on the conformations of the norditerpenoid alkaloid framework.

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Antibacterial and cytotoxic principles of *Commiphora glandulosa* stem bark resin

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Plant-derived medicines have been part of traditional healthcare in most parts of the world for thousands of years and there is an increasing interest in plants as sources of agents to fight microbial diseases and cancer (Farnsworth et al 1985). Given the alarming incidence of antibiotic resistance in bacteria of medical importance (Pezzuto 1997), there is a constant need for new and effective therapeutic agents. This study is part of an ongoing project to search for novel drugs from a vast array of medicinal plants from Botswana, which have not been studied extensively (Van Staden et al 2000). Chloroform and water extracts from *Commiphora glandulosa* resins (Burseraceae) were evaluated for their therapeutic potential as antimicrobial and anticancer agents using in vitro assays. Both chloroform and water extracts showed activity against Gram-positive bacterial test organisms such as *Bacillus subtilis* (NCTC 10073), *Staphylococcus aureus* (NCIMB 9518), *Clostridium perfringens* (NCTC8237) and multiple drug resistant strain *Staphylococcus aureus* (SA1199B), with MIC values ranging from 7.8 to 31.3 $\mu g\,mL^{-1}$. Both water and chloroform extracts were also cytotoxic against the murine cell line RAW264.7 macrophages and human leukaemia cell line U937 monocytes with IC_{50} values ranging from 15 to 20 $\mu g\,mL^{-1}$. The isolated active principle had an IC_{50} of 6.18 $\mu g\,mL^{-1}$ against cell line RAW 264.7 macrophages. Neither the crude extracts nor the pure compound showed activity against Gram-negative bacterial test strains ((*Esherichia coli* (NCTC9002), *Pseudomonas aerigunosa* (NCIMB10421), *Klebsiella aerogenes* (NCTC5055) and the fungal test organisms (*Candida albicans* (NCPF 3179) and *Aspergillus fumigatus* (NCPF 7097)). These findings support the use of *Commiphora glandulosa* resins by the rural communities in Botswana as antiseptic agent.

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Antibacterial and antioxidant cassane diterpenoids from *Mezoneuron benthamianum*

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Plants have been found useful in accelerating wound healing, a complex process involving the interplay of many biochemical and cellular mediators. Microbial infections and the presence of oxygyen free radicals are known impediments to wound healing. Notable among the microorganisms delaying or inhibiting wound healing are *Staphylococcus*, *Streptococcus* and *Pseudomonas* species (Hollinworth 1997). Any agent capable of eliminating or reducing the number of microorganisms present in a wound, as well as reducing the levels of ROS, may facilitate the wound healing process. A number of in vitro models are employed to assess relevant antimicrobial and antioxidant properties. *Mezoneuron benthamianum* Baill. (Caesalpinaceae) finds use in Ghanaian folk medicine for the treatment of skin diseases, wounds and dysentery. The petroleum extract of this plant shows antimicrobial and antioxidant activity. Investigation of this extract has resulted in the isolation of new cassane-type diterpenoids, R1, R2, R3, R5 and R9, from the air dried roots of *M. benthamianum*, along with ß-sitosterol and stigmastinone. The structures of these compounds were elucidated by spectroscopic studies using a combination of 1D and 2D NMR Spectroscopy and Mass Spectrometry (ESI). Assessment of minimum inhibitory concentrations using the micro dilution assay (Eloff 1998) was carried out for each compound in 96-well plates. Four of the compounds were active against several bacterial species (Table 1). The antioxidant activity was tested using the free radical DPPH (2, 2 diphenyl-1-picrylhydrazyl) (Cuendet et al 1997). Significant antioxidant activity was observed for two of the compounds. The results obtained suggest that the traditional use of the plant may be due to its antibacterial and antioxidant properties.

Table 1 Minimum inhibitory concentration (MIC) of compounds and standard antibiotics ($\mu g\,mL^{-1}$)

Bacterial sp.	R_1	R_2	R_3	R_5	R_9
SA 1199B (MRSA)	32.0	32.0	64.0	—	64.0
XU 212 (TetK)	64.0	32.0	128.0	—	128.0
RN 4220 (MSRA)	—	—	—	—	—
NCTC 4263 (S.a)	31.2	15.6	62.5	—	31.2
NCTC 10073 (B.s)	15.6	31.2	62.5	—	—
NCTC 775 (S.f)	—	125	250	—	125.0
NCIMB 1042 (P.a)	500	125	—	—	250.0
NCTC 7743 (M.f)	31.2	15.6	62.5	250	62.5

All MICs were determined in triplicate. —, MICs greater than 1000 $\mu g\,mL^{-1}$. M.f, *M. flavus*; B.s, *B. subtillis*; S.a, *S. aureus*; S.f, *S. feacalis; P.a, P. aeruginosa*; MRSA, Methicillin-resistant *S. aureus* and TetK, tetracycline-resistant *S. aureus* (Culture collections obtained from the Dept. of Pharmacy, King's College London and the School of Pharmacy, University of London). Tetracycline was used as positive control.

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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.

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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.

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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/