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Analysing raman images of pharmaceutical products by sample-sample 2D correlation spectroscopy

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Chemical images of solid dosage formulations (e.g. tablets) recently received keen interest in pharmaceutical industry. These images are produced from a very large number of vibrational spectra (typically several thousands). Imaging pharmaceutical formulations about which no information is provided a priori is a very complex task. To image a sample, one usually determines a wavenumber that is uniquely assignable to each component and then produce images that represent spatial distributions of each component based on the band intensities at these wavenumbers. This approach is called univariate imaging. However, as the spectra of the species present in an unknown sample are not available, this simplest form of imaging is not an option. Instead, one has to employ full spectra and mathematically determine those characteristic wavenumbers. Alternatively, one can image using full spectral range (this is known as multivariate imaging). An algebraic routine known as principal component analysis (PCA) is normally used as the first step in the analysis of such demanding matrices of spectral data. However, PCA results are not necessarily easy to understand and apply, as they are mathematical rather than physical concepts. Sample-sample (SS) 2D correlation spectroscopy is a method for data analysis that can be understood as a prestep to PCA. It is relatively simple to understand and apply and so far has been applied to solve various problems in analyses of highly overlapped vibrational spectra. Following upon recent introduction of sample-sample (SS) 2D correlation spectroscopy as a spectral selection tool for producing chemical images (Sasic et al 2003), we apply it here for imaging real-world pharmaceutical samples made of two, three and four components. The most specific spectra of those samples are found after multiplying the original data matrix by itself (covariance matrix). Chemical images of the components are subsequently produced in the univariate fashion by visually selecting the wavenumbers in the extracted spectra that are least overlapped. The images are obtained using no background information and represent spatial distribution of the active pharmaceutical ingredient, avicel, lubricant, or structure of a bead formulation. The performance of the SS 2D correlation spectroscopy is compared with PCA in terms of extracting most important spectral differences across the whole spectral sets, and in terms of determining number of the present species. In addition, SS 2D correlation spectroscopy is compared with another more complex algebraic approach concerning selection of the spectra. Both comparisons are found satisfactory and encourage use of simple SS 2D correlation routine as an initial approach for producing chemical images of pharmaceutical samples.

Sasic, S., Jiang, J.-H., Sato, S. et al (2003) *Analyst* **128**: 1097–1103

010
Derivative and ratio derivative spectrophotometric method for simultaneous determination of theophylline and guaifenesin in pharmaceutical formulation in comparison with HPLC method

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Theophylline (THP), a xanthine-based alkaloid, which relaxes smooth muscles and relieves bronchospasm, has a stimulant effect on respiration. Guaifenesin (GU), a mucolytic agent, is used as an expectorant. The association of these drugs might produce a synergy effect in therapy (Hardman et al 2003). THP has been marketed in combination with GU in pharmaceutical formulations used in the symptomatic treatment of bronchial asthma and other bronchospastic conditions. Although many chromatographic methods exist for the assays of both drugs (Koves 1995; Perez-Martinez et al 1996), there isn't any simple spectrophotometric method to determine THP and GU in the presence of each other that in this research has been proposed. Two methods are described for the determination of THP and GU in combined pharmaceutical syrup forms. These methods are based on the derivation of the main spectra. Derivative UV spectroscopy has been widely used as a tool for quantitative analysis, characterization, and quality control in agricultural, pharmaceutical, and biomedical fields. The first method that can be applied only for THP depends on the use of the second derivative spectra of the absorption spectrum of binary mixtures. The second derivative amplitudes at 291.6 nm were selected for the assay of THP. The second method is based on the use of the first derivative of the ratio-spectra obtained by dividing the absorption spectrum of binary mixtures by a standard spectrum of one of the compounds. The first derivative amplitudes at 286.2 and 231.7 nm were selected for the assay of THP and GU, respectively. Calibration curves were established at 5–50 $\mu g\,mL^{-1}$ for GU and 20–100 $\mu g\,mL^{-1}$ for THP. High-performance liquid chromatography was performed on a reversed-phase column using a mobile phase of methanol–water (40:60, v/v) (pH 3) with detection at 229 nm. Linearity was obtained in the concentration range of 5–75 $\mu g\,mL^{-1}$ for GU and 10–150 $\mu g\,mL^{-1}$ for THP. The relative standard deviations were found to be less than 4.5%, for between days and 5.0% for within days precision, indicating reasonable repeatability of both methods. Analytical recovery close to 95.1–103.4% shows the suitability of the method for determination in quality control. The detection limits for THP and GU were 0.95 and 1.82 $\mu g\,mL^{-1}$ by ratio-spectra derivative spectrophotometry and 0.59 and 0.86 $\mu g\,mL^{-1}$ by HPLC, respectively. The proposed methods were successfully applied to the determination of these drugs in laboratory-prepared mixtures and in syrup. Results show that this method is fast and economical in comparison with the more time-consuming HPLC method regularly used for formulation screening and quality control and can be used routinely by any laboratory possessing a spectrophotometer with a derivative accessory.

Hardman, J. G., et al (2003) *Goodman & Gilman's the pharmacological basis of therapeutics*. (CD-ROM), 10th edn, Mc Graw-Hill, pp 896–898
Koves, E. M. (1995) *J. Chromatogr. A* **692**: 103–119
Perez-Martinez, I., et al (1996) *J. Liq. Chromatogr. Rel. Technol*. 19: 1957–1966

011
Antimicrobial and antioxidant properties of extracts from Scottish plants

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Most medicines in use today can trace their origin to folk medicine. Scotland has a rich history of traditional medicine and was the site of some of the earliest medieval hospitals in Britain. Since the most prevalent illnesses during that era were infectious diseases caused by microbial organisms, it is probable that many of the medicines, usually plant extracts, used to treat patients had antimicrobial properties. Bioactivity-guided fractionation, isolation and structural elucidation of natural products is one of the successful methods in the eternal quest for novel medicinal compounds. We have initiated a programme to screen selected Scottish plants for antioxidant property, general toxicity and antimicrobial activity, and to isolate the bioactive compounds from these plants. This presentation will focus on the antimicrobial activity of some of the plant extracts tested to date. Plant seeds were ground and Soxhlet-extracted using solvent mixtures of increasing polarity. These extracts were tested for their antimicrobial activity using the checkerboard and resazurin assays. The seeds of the plants showing activity were then obtained in larger quantities and extracted on a larger scale, and using various separation techniques including TLC, PTLC, VLC, column chromatography and HPLC, compounds were isolated from the extracts and characterised by UV, FTIR, MS and extensive NMR spectroscopic data analyses. The DPPH assay method was adopted to evaluate the antioxidant activity for this study. This method utilises the fact that 2,2-diphenyl-1-picryl-hydrazyl (DPPH), a free radical showing characteristic absorption at 517 nm (purple), is decolourised by antioxidants. To quantify the degree of antioxidant potential of extracts, the degree of decolouration various concentrations of quercetin and trolox had on DPPH was obtained and tabulated and a graph obtained. This graph was used as the reference to compare the degree of antioxidant potential of the plant extracts. The main determinant for assessing in-vitro activity of antibiotics on microorganisms has been to determine the MIC. For this study the disc diffusion method, as well as the microwell method, has been utilised. Using aseptic techniques, 2 mL of the bacterial solution was transferred to 18 mL of agar in universal bottles. It was mixed thoroughly by shaking and then poured onto sterile petri dishes using aseptic technique. For the disc diffusion method, discs containing the extracts at a concentration of

1 mg mL^{-1} were placed on to the petri dishes using aseptic precautions. The petri dishes were labelled and placed in incubator at 37°C and were examined after 12, 24 and 36 h. Any inhibition zone was noted and compared with the positive controls, gentamicin and ciprofloxacin discs. Appropriate negative controls containing MeOH and blank discs were used. The microwell method used a modification of the checkerboard assay. The antimicrobial potency of the isolated compounds was evaluated. There were a number of plant extracts that showed antimicrobial activity against a number of pathogenic bacterial species, including MRSA, and also demonstrated significant antioxidant activity in comparison with the positive controls trolox and quercetin.

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In-vitro cytotoxic activity of *Kigelia pinnata* fruits

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The fruits of *Kigelia pinnata* DC (Bignoniaceae) are the most commonly used part of this plant, and in southern Africa anecdotal reports describe the use of the fruit to treat skin cancer but no specification has been found about the age of the fruit used (Hartwell 1968; Houghton 2001). Extracts of ripe (DF) and unripe fruits (FF) made with dichloromethane (DCM) and methanol (M) were tested for cytotoxicity using the SRB assay (Skehan et al 1990) on three different cell lines: C32 melanoma, SVK-14 keratinocytes and HF human fibroblasts. Six dilutions of each crude extract from 100 μg mL^{-1} to 0.05 μg mL^{-1} were tested in four replicates at different times of exposure. At 48 h (C32 and HF) and at 72 h (SVK-14) recovery (Rec) of the cells was tested by washing the extracts off from the cells and replacing by normal media, to investigate whether any effect was cytotoxic or cytostatic. Vinblastine sulfate(Vinb) for the C32 and HF cell lines and dithranol (Dithl) for the SVK-14 cells were used as positive controls. Two duplicate tests for each crude extract were performed and the IC50 was calculated using GraphPad-Prism software and results are shown in Table 1. The results show that the ripe fruit extracted with dichloromethane was the most cytotoxic against the C32 melanoma and the SVK-14 keratinocyte cell lines, although with SVK-14 cells all the crude extracts showed little activity. The selectivity and non-toxicity of the extracts was shown by the high IC50 values obtained with the human fibroblasts (HF). The data obtained with these preliminary investigation justify the traditional use of the fruit to treat skin problems, however further investigations are in progress. It appears that the fruits have to be ripe for the desired activity.

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Table 1 IC50 values (μg mL^{-1}) for *K. pinnata* fruit extracts against cell lines

Cell line	Extract	Exposure time			
		24 h	48 h	48 h Rec	144 h
C32	DF-DCM	> 100	> 100	42	38
	FF-DCM	> 100	> 100	100	> 100
	DF-M	> 100	> 100	> 100	> 100
	FF-M	> 100	> 100	> 100	> 100
	Vinb	30	3	0.5	0.1
HF	DF-DCM	> 100	> 100	> 100	> 100
	FF-DCM	> 100	> 100	> 100	> 100
	DF-M	> 100	> 100	> 100	> 100
	FF-M	> 100	> 100	> 100	> 100
	Vinb	38	37	30	12
72 h Rec SVK 14	DF-DCM	65			
	FF-DCM	> 100			
	DF-M	> 100			
	FF-M	> 100			
	Dithl	0.5			

Hartwell, J. L. (1968) *Lloydia* **31**: 71–170
Houghton, P. J. (2001) *S. Afr. J. Botany* **68**: 1420–1428
Skehan, P., et al. (1990) *J. Natl Cancer Inst.* **82**: 1107–1112

013
Investigation of some Thai medicinal plants used to treat cancer

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Six medicinal plants were selected because of their use in Thai traditional medicine to treat cancer (Table 1). All plant materials were extracted, according to the methods used traditionally, to obtain water and alcoholic extracts and the extracts were tested in-vitro for cytotoxicity against normal cell lines and cancer cell lines using the Sulforhodamine B (SRB) assay which assesses growth (Lin et al 1999). Four different types of cancer cell lines; COR-L23 (large cell lung carcinoma), MCF7 (human Caucasian breast adenocarcinoma), C32 (human amelanotic melanoma) and CACO2 (Human colon carcinoma), and one normal cell line, SVK-14 (human keratinocyte), were used. An ethanol extract of *A. baccifera* exhibited the strongest cytotoxicity against both cancer cell lines and normal cell lines. The effect on large cell lung carcinoma (COR-L23) of ethanol extract of *A. baccifera* showed the lowest IC50 (27.12 ± 1.40 μg mL^{-1} for 48 h exposure period and 19.95 ± 0.87 μg mL^{-1} for recovery period) (Table 1). It is possible that cytotoxic compounds are produced from the extracts only after first-pass metabolism. Therefore, all water extracts were tested after incubation with Cytochrome P450 enzyme systems as a model of this metabolic system. It was found that none of the plant extracts under investigation produced cytotoxic metabolites after incubation with CYP450. Since *A. baccifera* exhibited the highest potential, work to isolate and purify the bioactive compounds present is in progress.

Table 1 List of Thai medicinal plants and IC50 values against COR-L23 cell lines (n = 3)

Thai medicinal plant	Extract		IC50 (μg mL^{-1})
Ammannia baccifera Linn.	Alcoholic	Exposure	27.12 ± 1.40
		Recovery	19.95 ± 0.87
Family: Lythraceae	Water	Exposure	27.91 ± 3.07
Part used: whole plant		Recovery	26.46 ± 1.14
Canna indica Linn.	Alcoholic	Exposure	49.33 ± 7.97
Family: Cannaceae		Recovery	43.57 ± 4.97
Part used: rhizome	Water	Exposure	149.40 ± 2.64
		Recovery	138.00 ± 2.33
Mallotus philippensis	Alcoholic	Exposure	92.56 ± 1.56
Muell. Arg.		Recovery	83.03 ± 15.67
Family: Euphorbiaceae		Exposure	100.9 ± 3.75
Part used: stem		Recovery	87.11 ± 0.37
Clinacanthus nutans	Alcoholic	Exposure	161.30 ± 13.27
Lindau.		Recovery	74.67 ± 13.48
Family: Acanthaceae		Exposure	> 200
Part used: leaf		Recovery	> 200
Polygala chinensis	Alcoholic	Exposure	62.02 ± 12.43
Linn.		Recovery	57.86 ± 6.68
Family: Polygalaceae	Water	Exposure	> 200
Part used: whole plant		Recovery	185.50 ± 4.49
Acanthus ebracteatus	Alcoholic	Exposure	86.60 ± 7.43
Vahl.		Recovery	88.01 ± 5.86
Family: Acanthaceae	Water	Exposure	89.27 ± 6.19
Part used: whole plant		Recovery	90.35 ± 5.04

Lin, Z. X., Hoult, J. R. S., Raman, A. (1999) *J. Ethnopharmacol.* **66**: 141–150

014
Searching for anti-inflammatory properties in *Witheringia solanaceae* L'Her (Solancaeae)

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NF-κB is a transcription factor implicated in the regulation of many pro-inflammatory cytokines and related proteins that participate in inflammatory diseases

such as rheumatoid arthritis, asthma and inflammatory bowel disease (Karin et al 2004). In the search for new anti-inflammatory compounds targeting the nuclear factor-kappa B (NF-κB) cascade, *Witheringia solanaceae* was selected for bioassay-guided fractionation. The plant was included in a recent EU-funded program (AINP) that utilized various targets of the NF-κB activation cascade to identify new natural products that inhibit this factor. This plant was identified as one of the lead species and this work describes its detailed chemical and biological profiling. *Witheringia solanaceae* L'Her (Solanaceae) is a medicinal plant widely distributed from Mexico to Bolivia. It is known in Panama as Tinanguak'Gid and Diguima goi, where it is used as an anti-hypertensive remedy and for general pain (Caballero-George et al 2001). In Mexico the plant is known as merengena and the fruit is eaten and prepared as "salsa". It is given to anemic persons and to treat skin problems such as fungal infections and acne (Hersch-Martínez et al 2000). The powdered leaves of *W. solanacea* were exhaustively extracted by soxhlet increasing the polarity of solvents. Different chromatographic techniques were used for the fractionation of crude extracts and isolation of compounds. A luciferase assay was performed to test the biological activity of fractions and compounds. HeLa cells were stably transfected with a luciferase reporter gene controlled by the IL-6 promoter (one of the target genes for activated NF-κB). PMA was used as a stimulant of NF-κB at 50 ng mL^{-1} for 7 h. Hexane and acetone extracts were active at 100 μg mL^{-1}, at this concentration the chloroform extract was toxic. However, it was decided to work-up the chloroform extract because of the chemical complexity and the higher yield of this extract. Three compounds were isolated from the chloroform extract. Compounds 1 and 3 were isolated by VLC, silica gel column and preparative TLC, while the isolation of compound 2 was achieved by VLC, SP-extraction and preparative TLC. Compound 1 was identified as physalin B using 1D and 2D Nuclear Magnetic Resonance (NMR) experiments on a Bruker Advance 500 NMR Spectrometer. Compounds 2 and 3 are physalin type compounds, and their structures are currently under elucidation. Compounds 1 and 2 were 100% toxic, but compound 3 showed a reduction to 8.6% of the positive control at 100 μg mL^{-1} and no cytotoxicity was observed.

Caballero-George, C., et al (2001) *Phytomedicine* **8**: 59–70
Hersch-Martínez, P., et al (2000) INAH 23–24
Karin, M., et al (2004) *Nat. Rev.* **3**: 17–26

015
Antimicrobial activity of selected Botswana medicinal plants

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There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases (Rojas et al 2003). Another big concern is the development of resistance to the antibiotics in current clinical use. Plants are of constant interest as a source of antimicrobial agents (Chariandy et al 2000) particularly as plant derived medicines have been part of the traditional healthcare in most parts of the world for thousands of years. In Botswana, like in other developing countries, medicinal plants still represent the main therapeutic tool in traditional medicine (Van Staden et al 2000). Indigenous medicines used in Botswana have not yet been studied extensively; thus the antimicrobial activity of two medicinal plants sourced from herbalists in Botswana is reported. Chloroform and water extracts of *Commiphora glandulosa* (Burseraceae) and *Clerodendrum uncinatum* (Verbenaceae) were evaluated for their therapeutic potential (i.e. antimicrobial compounds) against a selection of Gram-positive (wild type *Staphylococcus aureus* NCIMB 9518, multiple drug resistant strains of *Staphylococcus aureus* XU212, 1199B London School of Pharmacy), *Bacillus subtilis* NCTC 10073, *Clostridium perfringens* NCTC 8237) and Gram-negative (*Escherichia coli* NCTC 9002, *Pseudomonas aerogenosa* NCIMB 10421, *Klebsiella aerogenes* NCTC 5055) bacteria and fungi (*Candida albicans* NCPF 3179, *Trichophyton tonsurans* NCPF 995, *Aspergillus fumigatus* NCPF 7097), employing the agar-well diffusion, TLC-bioautography and microdilution assays. Chloroform and water extracts of *Commiphora glandulosa* showed significant activity against Gram positive microorganisms, including the multiple drug resistant strains of *S.aureus*. Active crude extracts exhibited minimum inhibitory concentrations of 7.8–500 μg mL^{-1} against Gram-positive bacteria with the microdilution assay. Of the two plants, the highest activity was observed in the extracts of *Commiphora glandulosa* (7.8 μg mL^{-1}) against *Clostridium perfringens*. None of the extracts showed activity against either the Gram-negative microorganisms or fungi. The folk-claims on the use of these crude drugs against infectious diseases were substantiated by the present observations. Isolation of active constituents is in progress in our laboratories.

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Chariandy, C. M., et al (2000) *J. Ethnopharmacol.* **64**: 265–270
Rojas, R., et al (2003) *J. Ethnopharmacol.* **88**: 199–204
Van Staden, J., et al. (2000) *J. Ethnopharmacol.* **71**: 281–292

016
Anti-diarrhoeal activity of *Trichodesma indicum* (Linn.) R. Br. root extract in rats

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Trichodesma indicum (Linn.) R. Br. (Boraginaceae) is a hispid, erect or diffuse annual herb with single pale blue flower, changing to pink or white. The herb is found as a weed throughout the greater part of India. In folk medicine, the root is pounded into a paste and is applied to reduce swellings, particularly of joints; the extract is given to children for diarrhoea, dysentery and fever. Roots used in this study were collected during the months of May and December 2002 from Road Maurvai Forest, in the Cuddalore district, Tamilnadu, South India. The plant was identified and a voucher specimen has been retained. The powdered roots were extracted successively with hexane chloroform and ethanol at room temperature. After exhaustive extraction, the ethanol extract was concentrated under reduced pressure at 50–55°C. A brownish black residue was obtained (yield 5.4% w/w with respect to the dry starting materials), and kept in a desiccator for further use. The extract at the different doses (100, 200 and 400 mg kg^{-1}) were suspended in 1% w/v carboxy methyl cellulose for administration to animals. Antidiarrhoeal activity was evaluated in Albino Wistar rats of either sex weighing 160–240 g were used. The ethanol extract (100, 200 and 400 mg kg^{-1}, p.o.) inhibited normal defaecation and castor-oil-induced diarrhoea (Awouters et al 1978) in a dose-dependent manner. The ethanol extract (400 mg kg^{-1}, p.o.) was found to cause significant inhibition of 70.58% in serotonin-induced diarrhoea (Doherty 1981). Pre-treatment with ethanol extract (400 mg kg^{-1}, p.o.) showed significant inhibitory effect against PGE_2 induced intestinal fluid accumulation in rats. The ethanol extract (100, 200 and 400 mg kg^{-1}, p.o.) also decreased gastrointestinal motility after charcoal meal administration in Wistar rats, when compared with atropine sulphate (0.1 mg kg^{-1}, i.p.). The above observations suggest that the ethanol extract of *Trichodesma indicum* root possessed significant anti-diarrhoeal activity in experimental animal models. These results may provide a basis for the use of *Trichodesma indicum* in non-specific diarrhoea, as a folk medicine.

Awouters, F., Niemegeers, C. J. E., Lenaerts, F. M., et al (1978) *J. Pharm. Pharmacol.* **30**: 41–45
Doherty, N. S. (1981) *Br. J. Pharmacol.* **73**: 549–554