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POSTER SESSIONS

SESSION 1
Analytical Chemistry

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Stability-indicating high-performance liquid chromatography method for the determination of erdosteine in bulk and pharmaceutical dosage forms

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Objectives Erdosteine (Erdotin), 2-((2-oxothiolan-3-yl)carbamoylmethylsulphanyl)acetic acid, is a thiol derivative developed for the treatment of chronic obstructive bronchitis, including acute infective exacerbation of chronic bronchitis. Erdosteine contains two blocked thiol groups which are released following first-pass metabolism (Dechant and Noble 1996). To our knowledge, no article related to a stability-indicating chromatographic determination of erdosteine in pharmaceutical dosage forms has been published in the literature. The International Conference on Harmonisation (ICH) guideline entitled *Stability testing of new drug substances and products* requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance (ICH 2003). The aim of this work was to develop a stability-indicating high-performance liquid chromatography (HPLC) method for erdosteine and related impurities for assessment of the bulk drug and the stability of its dosage form.

Methods A sensitive and reproducible HPLC method is described for the quantitative determination of erdosteine in the presence of its degradation products. The method was based on HPLC separation of the drug from its degradation products on a C_{18} Perfectsil column (5 μm, 25 cm × 4.6 mm, inner diameter) at ambient temperature using a mobile phase consisting of 20 mM phosphate buffer/heptane sulphonic acid/acetonitrile (80:1:20, by vol.), pH-adjusted to 3.0 ± 0.2 with orthophosphoric acid. Flow rate was 1.0 mL min^{-1} with an average operating pressure of 180 kg cm^{-2} and t_r was found to be 5.62 ± 0.02 min.

Results Quantitation was achieved with UV detection at 254 nm based on peak area with linear calibration curves in the concentration range 10–100 μg mL^{-1}. This method has been successively applied to pharmaceutical dosage formulation. The drug content was found to be 100.25% with a percentage relative SD (RSD) of 0.57. The limit of detection and limit of quantitation values were found to be 0.10 and 0.50 μg mL^{-1}. The method was found to be precise with percentage RSD values of 1.51 and 1.04 respectively for intra- and inter-day precision studies. Statistical evaluation of the results was performed with regard to accuracy and precision using Student's *t* test and *F* ratio at the 95% confidence level.

Conclusions The developed method is a simple, accurate, reproducible, and stability-indicating quantitative determination of erdosteine in a pharmaceutical formulation, without interference from the excipients and in the presence of its acidic, alkaline, oxidative, and photolytic degradation products (Table 1). The chromatographic method was validated according to ICH guidelines. Statistical tests indicated that the proposed method reduces the duration of analysis and appears to be equally suitable for routine analysis in pharmaceutical formulation in quality-control laboratories, where economy and time efficiency are essential. This study separates the drug from its degradation products, and hence is a typical example of a stability-indicating assay.

Table 1 Summary of degradation parameters

Condition	Time (h)	Recovery (%)	Degradation (%)	t_R of degradants
Acid 0.1 M	1.0 hours	86.23	20.79	6.35, 6.29
Base 0.1 M	1.0 hours	89.65	11.35	7.25
H_2O_2 10% (w/v)	2.0 hours	77.20	22.48	4.22, 4.54
UV	45 days	99.01	–	4.18

Dechant, K. L., Noble, S. (1996) *Drugs* **6**: 875–881

International Conference on Harmonisation (2003) *Stability testing of new drug substances and products*. ICH-Q1A (R2). International Conference on Harmonisation

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Validation of a high-performance liquid chromatography method for colistin sulphate and related substances in colistin tablets

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Objectives Colistin sulphate is an antibiotic consisting of various polymyxin components. Historically the content specification has been defined in international units (IU) per mg, measured using a microbiological assay method. The European Pharmacopoeia recently published a revised monograph for colistin sulphate in which the microbiological assay was replaced by high-performance liquid chromatography (HPLC). The objectives were to investigate the suitability of the HPLC assay method for the colistin sulphate pharmaceutical substance monograph (European Directorate for the Quality of Medicines 2007) for application to colistin tablets and to determine whether the method could be used as a related substances test. Currently, colistin tablets are labelled in international units, not by weight. This presents a significant challenge when attempting to compare an HPLC method with a biological method because the HPLC method determines an *amount* whereas the biological method determines an *activity* and a correlation between the two cannot be guaranteed.

Methods Samples of colistin tablets (Colomycin®, 1500000 IU; Forest Laboratories) were extracted using 40 mL of water (ELGA 18 MΩ resistivity) for 20 minutes, diluted to 50 mL with acetonitrile (Rathburn Chemicals), mixed well and filtered. The filtrates were analysed by HPLC using an Agilent 1100 high-performance liquid chromatograph; a C_{18} column maintained at 30°C (SunFire 3.5 μm, 15 cm × 0.46 cm; Waters Corporation); a mixture of 22 volumes of acetonitrile and 78 volumes of a solution prepared by dissolving 4.46 g of anhydrous sodium sulphate in 900 mL of water, adjusting to pH 2.4 with dilute orthophosphoric acid and diluting to 1000 mL with water as mobile phase; a flow rate of 1.0 mL/minute; an injection volume of 20 μL and UV detection at 215 nm. Reference standards of colistin sulphate were obtained from the European Directorate for the Quality of Medicines.

Results The chromatography of sample solutions was satisfactory and similar to that of the reference standards. All secondary peaks detected were adequately resolved from the principal peaks (polymixins E1, E2, E3, E1-I and E1-7MOA) and could be readily integrated. There was no evidence of excipient interference. The repeatability of the method was satisfactory for related substances (consistent results obtained for three replicate samples). The results of the tablet samples (by area normalization) were all within the colistin sulphate monograph limits (any impurity maximum 4.0%; total impurities maximum 23.0%). The repeatability of the method was satisfactory for assay (relative standard deviation of five replicate sample results was 1.16%). The linearity of the method was satisfactory for both samples and standards over the concentration range 0.02–0.05% w/v, representing 50–120% of the expected sample concentration (r^2 = 0.9997 and 0.9978, respectively) and the accuracy of the method was satisfactory (determined recovery was 108.5%, based on a label claim of 75 mg).

Conclusions This study has shown that it is possible to determine the content and purity of colistin tablets BP using HPLC. This is a step forward in improving the accuracy, precision and accessibility of the Pharmacopoeial assay method and it also allows a measurement of impurities and degradants, which is a not possible using the microbiological method.

European Directorate for the Quality of Medicines (2007) *European pharmacopoeia*, 6th edn, **2**: 1615–1617

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Determining active pharmaceutical ingredient solubility in hydrofluoroalkane propellants using nuclear magnetic resonance spectroscopy

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Objectives The objective of this investigation was to develop a rapid new etechnique to determine active pharmaceutical ingredient (API) solubility in

hydrofluoroalkane (HFA) propellant using nuclear magnetic resonance (NMR) spectroscopy. The extent to which an API is soluble in the HFA propellants can dictate whether the formulator decides to develop a solution- or suspension-based propellant metered-dose inhaler (pMDI) formulation. As a consequence of the high volatility of the propellants, traditionally pressurized filtration systems in combination with liquid chromatography quantification methodology are used to determine the solubility of API in propellant. The NMR spectroscopy method was developed as a rapid alternative technique.

Methods A suspension of beclomethasone dipropionate (BDP) in HFA-227 was prepared in an NMR tube with a J Young valve (typical volume 1–2 mL). The signal from the HFA solvent protons can be partially removed by using standard solvent-suppression techniques, while the spectra can be quantified by using an external standard, known as the ERETIC method. In this case a 'standard' signal is generated electronically and can be referenced externally against the normal NMR standards. Since solution-state NMR only detects signal from APIs in solution, the solubility of the API in a saturated suspension can be readily determined. All NMR data was recorded on a Bruker Avance 500 using a 5 mm BBI probe. Data was recorded under quantitative conditions using a standard pre-saturation pulse sequence to suppress the solvent signal with an ERETIC pulse for quantification. All NMR data were recorded at 290 K. For comparison purposes, the solubility of BDP was determined using a pressure filtration technique. To assess the solubility limit of BDP in HFA-227, pMDIs containing four different concentrations of BDP were prepared (0.005, 0.025, 0.05 and 0.075% w/w). The samples were then filtered under pressure using a 0.2 μm PTFE membrane filter located in a leak-tight housing of bespoke design. The filtrate was collected in a receiving vessel, and the propellant then vented, leaving the dissolved drug behind for reconstitution into a suitable solvent. The API content was then assayed using liquid chromatography with mass spectrometry detection.

Results The solubility of BDP was determined to be approximately 120 μg/mL using NMR and demonstrates that the technique is sensitive enough to be applied to drug products that are sparingly soluble in HFA propellant. Further evaluation has demonstrated that the technique is capable of quantitation down to levels of approximately 10 ppm. Results obtained from the liquid chromatography-mass spectrometry analysis of the pressure filtration samples showed that maximum solubility was observed in the 0.05% w/w suspension of BDP, which is comparable to the NMR-derived value of 120 μg/mL.

Conclusions Comparison of data generated by NMR and pressure filtration demonstrates that similar results were obtained using both techniques, and that the NMR solubility method may provide a rapid alternative to pressure filtration for determination of API solubility in HFA propellants.

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New spectrophotometric estimation of tinidazole tablets using ibuprofen sodium as a hydrotropic solubilizing agent

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Objectives Organic solvents are often used for spectrophotometric analysis of poorly water-soluble drugs. These solvents have the disadvantages of high cost, volatility and toxicity, as well as being hazardous. To minimize these problems a new, simple, rapid, economic, accurate and reproducible method of analysis has been developed using the hydrotropic solubilizing agent ibuprofen sodium to negate the use of organic solvents. The aim of the present study was to explore the potential of ibuprofen sodium as a hydrotropic solubilizing agent in the spectroscopic analysis of tinidazole.

Methods Solubility of tinidazole (a poorly water-soluble drug) was determined in distilled water and 1.5 M ibuprofen sodium solution. Tinidazole was more than three times more soluble in 1.5 M ibuprofen sodium solution than in distilled water. For assay purposes tinidazole was solubilized from its tablet form using a 1.5 M ibuprofen sodium solution. Tinidazole 100 mg was transferred to a 25 mL volumetric flask and 20 mL of 1.5 M ibuprofen sodium solution added, the drug dissolved and the solution made up to volume with distilled water. This solution was diluted with distilled water to prepare solutions containing 5, 10, 15, 20 and 25 μg/mL drug. The absorbance of these solutions was measured at 318 nm. For spectrophotometric analysis of two tablet formulations, I and II, containing tinidazole, 20 tablets were weighed and powdered. Powder equivalent to 100 mg tinidazole was transferred into a 25 mL volumetric flask containing 20 mL of 1.5 M ibuprofen sodium and shaken to solubilize drug and made up to volume using distilled water. The solution was filtered through Whatman filter paper (no. 41) and diluted with distilled water and absorbance recorded at 318 nm. Recovery experiments were performed to determine the accuracy, reproducibility and precision of the method.

Results The percentage label claims in the case of the proposed analytical technique, 98.73 ± 1.003% (formulation I) and 99.42 ± 1.222% (formulation II), were very close to 100%, indicating the accuracy of the method. The accuracy of the method was further confirmed by the values of percentage label claims (99.58 ± 1.534% for formulation I and 100.32 ± 0.939% for formulation II) obtained by the Indian Pharmacopoeial method of assaying for tinidazole. The low values of standard deviation, percentage coefficient of variation and standard error further validated the proposed analytical method. The percentage recoveries (using the proposed method of analysis) were 98.72 ± 0.942 and 99.62 ± 1.201% for formulation I and 101.34 ± 1.667 and 99.89 ± 0.337% for formulation II, indicating the method's accuracy. The low standard deviation, percentage coefficient of variation and standard error values further validated the proposed analytical method.

Conclusion It is concluded that the proposed method of analysis of tinidazole using iburopfen sodium is novel, simple, cost-effective, environmentally friendly, safe, accurate and reproducible and can be employed for the analysis of tinidazole in tablets avoiding the use of organic solvent. Other poorly water-soluble drugs with a λ_{max} above 300 nm may also be examined for solubility enhancement using 1.5 M ibuprofen sodium solution.

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Simultaneous reverse-phase high-performance liquid chromatography estimation of cefixime and cloxacillin from tablet dosage forms

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Objectives A literature survey revealed that there is no simple, precise, rapid and selective high-performance liquid chromatography (HPLC) method for the combination of cefixime and cloxacillin in any dosage form. The proposed method was developed and validated for simultaneous determination of cefixime and cloxacillin by HPLC, which is simple, precise, rapid and selective.

Methods A new reversed-phase HPLC method was developed and validated for the simultaneous determination of cefixime and cloxacillin in tablets. The separation was achieved by using a Jasco PU-2080 Plus intelligent HPLC on a HiQ sil C_8 analytical column (4.6 mm × 250 nm) using acetonitrile and tetrabutyl-ammonium hydroxide (45:55 v/v, pH 4.00 ± 0.05) as a mobile phase at a flow rate of 1 mL/minute. Detection was carried out using a UV detector at 225 nm. The developed and validated method was successfully applied for the quantitative analysis of cefi-xl® tablets. The total chromatographic analysis time per sample was about 15 minutes, with cefixime, verapamil (internal standard) and cloxacillin eluting at retention times of about 5.7, 8.5 and 11.9 minutes, respectively. No spectral or chromatographic interferences from the tablet excipients were found.

Results The calibration plots were linear and obeyed the Beer–Lambert law. The method was validated for precision, accuracy, linearity, reproducibility and robustness and statistical comparison was peformed by analysis of variance.

Conclusions The developed HPLC technique is precise, specific, accurate and stable. Statistical analysis proves that the method is reproducible, selective and suitable to be applied for analysis of cefixime and cloxacillin in commercial pharmaceutical dosage forms for routine quality control application.

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Use of simple standards for model transfer in quantitative analysis of intact tablets by near-infrared spectroscopy

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Objectives To transfer spectra measured on a slave instrument to a model created on a master instrument using simple pure compounds or mean sample spectra as the transfer standard.

Methods Reflectance near-infrared (NIR) spectra of 116 Sterwin® 500 mg paracetamol intact tablets (29 batches, 76.5–91.6% m/m active, nominal diameter 12.9 mm) were measured on three different FOSS NIRSystems instruments. Reference values for the active were measured according to the British Pharmacopoeia 2008. Instruments: A, XDS Masterlab spectrometer; B, XDS spectrometer fitted with a rapid content analyser (RCA) and C, 6500 monochromator fitted with a RCA. The following transfer standards were used; avicel PH101, benzoic acid, methylparaben, paracetamol, sucrose, mean sample spectrum (38 paracetamol tablets), mean paracetamol tablets (other brands) and six different pharmaceutical tablets. Two containers, a 12 mm diameter vial and a 50 mm diameter cell, were used to measure the powders. Spectra of tablets measured on instruments B and C were corrected using the difference spectrum of

the transfer standard measured on A and B or C respectively. These corrected spectra were then used to predict the paracetamol content using a model developed on A.

Results A three-factor partial least squares regression (PLSR) calibration model (1100–2498 nm) using SNV + first-derivative spectral pre-treatment on instrument A gave an RMSEP of 0.57% m/m. Direct transfer of spectra from instrument B gave an RMSEP of 0.81% m/m. Corrected spectra gave slightly better results: for example, avicel PH101 in vial (0.95% m/m), avicel PH101 in cell (0.60% m/m), paracetamol in vial (1.14% m/m), sucrose in vial (0.58% m/m), sucrose in cell (0.81% m/m) and mean sample spectrum (0.56% m/m). Transfers between different instrument types (A and C) were less successful: direct transfer (0.97% m/m), avicel PH101 in vial (0.75% m/m), avicel PH101 in cell (1.60% m/m), paracetamol in vial (0.54% m/m), sucrose in vial (1.07% m/m) and sucrose in cell (1.03% m/m). However, correction using the mean sample spectrum was best (0.52% m/m). Many factors influenced the success of transfer. Model selection was important; for example, increasing the level of smoothing during spectral pre-treatment generally improved transfer. Matching the transfer standard dimensions to that of the paracetamol tablets was also an important factor. In general the transfer standard needs to closely match the sample both chemically and physically.

Conclusions Transfer using simple pure compounds, while useful, was not to be preferred over mean sample spectrum correction.

Biologics

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Synthesis and evaluation of an affinity-based probe for fibroblast-activation protein α (seprase) activity

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Objectives Fibroblast-activation protein a (FAPα; also known as seprase) is a 170 kDa serine gelatinase and prolyl dipeptidyl peptidase that consists of two identical N-glycosylated 97 kDa subunits embedded in and integral to the cell membrane. FAPα is expressed during tumour cell invasion at the invasive fronts of melanoma cells and breast carcinoma cells, in gastric carcinoma and also in rheumatoid arthritis. FAPα belongs to a family of post-proline-cleaving proteases, many of which share a high degree of homology and/or substrate specificity. This is particularly true of dipeptidyl peptidase IV (DPP-IV), another therapeutically important protease. The purpose of this study was to design, synthesize and evaluate an affinity-based probe for the selective disclosure of FAPα activity in biological milieux where other closely related peptidase activities are present.

Methods The affinity-based probe, Bio-PEG-T-S-G-$P^P(OPh)_2$, was synthesized by a combination of solid- and solution-phase synthesis. The amino acid sequence was based on previous work by our group which indicated that Gly-$Pro^P(OPh)_2$ was a potent irreversible inhibitor of both DPP-IV and FAPα (Gilmore et al 2006), and a recent report indicating that FAP could cleave extended substrates (Edosada 2006), thus permitting the design of selective inhibitors which do not target closely related peptidases such as DPP-IV. The inhibitor was evaluated by continuous fluorimetric assay against FAP, DPP-IV, DPP2 and DPP7. Labelling of peptidases was conducted as described by Gilmore et al (2007) using standard electrophoresis and western-blotting methodologies. Labelled peptidases were detected by streptavidin-HP chemiluminescence on photographic film.

Results The inhibitor probe Bio-PEG-T-S-G-$P^P(OPh)_2$ was found to be a moderately potent but highly selective inhibitor of FAPα, having an overall second-order rate constant (k_i/K_i) of 3.8×10^3 M^{-1} min^{-1}. Importantly, Bio-PEG-T-S-G-$P^P(OPh)_2$ exhibited no inhibitory activity against DPP-IV, DPP2 or DPP7. The probe was subsequently used to label recombinant FAPα and shown to be directed by the active site, since incorporation of the probe was blocked by pre-incubation of the enzyme with pefabloc.

Conclusions In this report we detail the synthesis of a moderately potent, selective affinity-based probe for the disclosure of FAPα activity in complex biological milieux even where closely related peptidase activities are present. This probe may have utility in further elucidating the role of this peptidase in diseases such as cancer and rheumatoid arthritis.

Edosada, C. Y. et al (2006) *FEBS Lett*. **580**: 1581–1586
Gilmore, B. F. et al (2006) *Biochem. Biophys. Res. Commun*. **346**: 436–446
Gilmore, B. F. et al (2007) *Biochem. Biophys. Res. Commun*. **347**: 373–379

Chemistry

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Photothermal microspectroscopy: a new technique for spatially differentiating between crystalline and amorphous materials

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Objectives This study demonstrates the ability of photothermal microspectroscopy (PTMS), a new technique (Hammiche et al 2004), to differentiate between both the physical and chemical nature of a model GlaxoSmithKline drug in its amorphous and crystalline forms.

Methods The model GlaxoSmithKline drug was used as received in its crystalline form. The amorphous form was prepared using a Büchi Mini-Spray Dryer B-290. Compacts of the samples were formed using a 13 mm infrared sample press with an applied pressure of 1 ton. PTMS was performed by interfacing a Caliber AFM equipped with a Wollaston wire thermal probe to a Bruker FTIR spectrometer. Local thermal analysis experiments were conducted with an underlying heating rate of 10°C s^{-1} from 60 to 200°C. Infrared spectra were collected using 200 scans and a resolution of 8 cm^{-1}, across the range 4000 to 500 cm^{-1}. Modulated-temperature differential scanning calorimetry (MTDSC) and attenuated total reflectance-Fourier transform infrared spectroscopy were used to validate the PTMS results.

Results PTMS demonstrated its ability to distinguish between the pure amorphous and crystalline surfaces. The topography of the crystalline and amorphous surfaces showed clear differences although it was not possible to state which surface represents which material from topography alone. Local thermal analysis, whereby the temperature of the tip is raised and the temperature of penetration noted (Royall et al 2001), was able to discriminate on the basis of the glassy or melting behaviour of the system. This agrees with the MTDSC response seen. For the first time we were also able to differentiate via the photothermal infrared spectra using PTMS, shown in Figure 1, with spectral differences noted in terms of peak broadening and disappearance in the 3500–2000 cm^{-1} region. This broadening is due to non-specific intermolecular bonding in the amorphous material.

Conclusions PTMS was shown to be a highly promising new technique for determining and differentiating between pure amorphous and crystalline materials at precise topographic locations. Subsequent work is focused on mixed systems and results to date are highly encouraging.

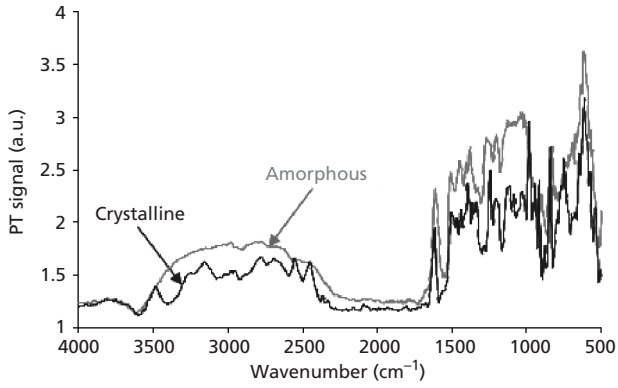

Figure 1 Photothermal IR spectra of the amorphous and crystalline samples.

Hammiche, A. et al (2004) *J. Microsc.* **213**: 129–134
Royall, P. G. et al (2001) *J. Phys. Chem. B* **105**: 7021–7026

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Synthesis, characterization, *in vitro* hydrolysis and biological evaluation of amino acid methyl ester conjugates of valproic acid: a prodrug approach

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Objectives Valproic acid (VPA) is an extensively used anti-epileptic drug for the treatment of various kinds of epilepsies and administered orally several times a day due to short half-life. It has been proven to possess the life-theatening side effects of hepatotoxicity, teratogenicity and gastric irritation (Sobol et al 2004, Bryant and