SESSION 2

Analytical Chemistry

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Identification of counterfeit Cialis tablets by Direct Analysis in Real Time (DART) time-of-flight mass spectrometry

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Objectives To use Direct Analysis in Real Time (DART) time-of-flight mass spectrometry (TOF MS) to analyse the film coats and cores of authentic and counterfeit Cialis tablets to determine the ability to quickly and effectively differentiate them with no, or minimal, damage of the tablet.

Methods Authentic samples of Cialis tablets were purchased from pharmacies in the London area. Samples of known counterfeit Cialis tablets were provided by the Korea Food and Drug Administration, Busan, Korea. A JEOL AccuTOF LC mass spectrometer equipped with a DART source was used. The DART source was operated with a gas flow of 1.5 L min^{-1} with temperatures ranging from 100–250 °C. Tablets were individually held in front of the DART source using forceps for approximately five seconds to analyse the film coats. To analyse the cores of the tablets, a capillary tube was used to scrape the surface of the core of the tablet (< 5% damage to the surface) and the powder on the tube was presented to the DART source. Mass calibrators comprising pure polyethylene glycol (PEG) 600 on capillary tubes were presented to the DART source in between each tablet sample.

Results The tablets only had to be waved in front of the source to get spectra. Good chemical ionisation mass spectra for the film coat were obtained with source temperatures of 100–150 °C, but the coat bubbled at 200 °C. The authentic tablets all showed a peak at 236 ([triacetin + NH_4]$^+$, typical measured accurate mass 236.1120) and 159 ([$C_7H_{10}0_4$ + H]$^+$, a triacetin fragment, typical measured accurate mass 159.0657) showing the presence of triacetin (one of the components of the film coat), whereas none of the counterfeit tablets examined showed the presence of triacetin. Reconstructed ion chromatograms (RICs) also easily demonstrated the lack of triacetin in the counterfeit tablets. When the scrapings of the tablets were presented to the source, good quality chemical ionisation mass spectra were obtained for the authentic Cialis tablets with large peaks at typically 390.1412, 159.0657 and 145.0458 (corresponding to [tadalafil + H]$^+$, the [triacetin fragment + H]$^+$ and a [polysaccharide fragment + H]$^+$ respectively). None of the counterfeit samples of Cialis contained tadalafil; they gave instead a large peak at typically 475.2073 due to sildenafil. These counterfeit tablets were therefore manufactured with sildenafil as the active pharmaceutical ingredient instead of tadalafil so that they did have clinical effect. Presumably it is easier to get illicit supplies of sildenafil than tadalafil. RICs obtained by sequentially putting tablets in the source separated by PEG 600 mass calibrators and using ions at 371.3194 (dioctyl adipate), 390.1479 (tadalafil), 391.2844 (dioctyl phthalate), 459.2731(PEG 600) and 475.2153 (sildenafil) clearly differentiated the authentic from counterfeit preparations within a few minutes.

Conclusions DART TOF MS can easily differentiate authentic and counterfeit Cialis tablets from their film coat and core compositions. The technique is minimally destructive, has high resolution and gives accurate mass measurements to allow molecular formulae of the ingredients to be identified within minutes.

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The identification of counterfeit and sub-standard ibuprofen tablets by near-infrared spectroscopy

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Objectives To develop a near-infrared spectroscopic (NIRS) method to identify counterfeit and substandard ibuprofen tablets by using calibration curves generated from using intact and crushed tablets with the general quantitative NIRS procedure developed by Shek et al (2006).

Methods Ibuprofen tablets 200 mg BP (nine different brands) were purchased from local community pharmacies. Tablets from each brand were crushed and either ibuprofen or microcrystalline cellulose added to give a total of 99 powdered samples with a wider ibuprofen concentration (17.63–87.25% m/m) than the original tablets. Powdered samples were scanned in Waters 4 mL glass vials in triplicate and the mean reflectance spectrum for each powder was calculated. Both sides of intact tablets (10 from each brand) were scanned twice, rotating the tablet through 90°, to give four spectra from which the mean reflectance spectrum for each tablet was calculated. A FOSS NIRSystems 6500 NIR spectrometer using a Rapid Scanning Analyser (RCA) over the range 1100–2500 nm was used with Vision 2.51 software to control the spectrometer and manipulate the data. The same ceramic reference standard was used, and 32 repeat scans were acquired for each spectrum.

Results The nine brands of ibuprofen tablets 200 mg had mean weights between 268.48 mg and 567.16 mg (74.49 – 35.26% m/m ibuprofen respectively, mean 48.48% m/m). The reference value for each tablet (ibuprofen % m/m) was taken as the label claim of the tablet (200 mg) divided by its weight. Of the 99 powder spectra, two were found to be outliers (> 3 standard deviations from the regression line) and omitted from further consideration. The remaining 97 spectra were split into a calibration set (58) and a validation set (39) chosen randomly to give a random selection of brands and concentrations of ibuprofen. Similarly, a calibration set (56 spectra) and a validation set (34 spectra) were chosen from the 90 mean tablet spectra. The sets of spectra were then used in various partial linear squares regression (PLSR) models (using The Unscrambler software). The best model was a three component PLSR model using the original spectra and multiplicative scatter correction. The regions 1420–1560 nm and 1850–2000 nm were removed to prevent any water present distorting the model. This model gave calibration and validation graphs with slopes of 0.993 and 0.971, respectively, and a root mean standard error of prediction of 0.844 and 1.39% m/m, respectively. There was no advantage in using powdered tablets or in using powders with an extended ibuprofen concentration range.

Conclusions The NIRS method can be applied to the non-destructive analysis of intact single ibuprofen tablets, without the need to manufacture calibration standards or the use of reference chemistry, with a root mean standard error of prediction of 1.39% m/m.

Shek, Y. H. M., et al (2006) *J. Pharm. Pharmacol*. **58** (Suppl.): A3

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Reverse phase-high performance liquid chromatography method for the analysis of atorvastatin in tablets and nanoemulsion formulation

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Objectives Many HPLC methods have been developed for the analysis of atorvastatin (AT) in bulk drug and tablet formulations (Chaudhari et al 2007) as well in self-microemulsifying mixture for in-vivo study (Shen et al 2006), but to date no method has been developed for the analysis of AT in the nanoemulsions formulation. Therefore in this study an attempt was made to develop a rapid, sensitive and effective method for the analysis of AT in the nanoemulsion formulation.

Methods For the method development Shimadzu model HPLC equipped with LiChrospher100 column (5 μm, RP-18, 250 × 4.6 mm), a quaternary LC-10A VP pump, UV/VIS detector SPD-10AVP, rheodyne injector fitted with a 20 μL loop and Class-VP 5.032 software was used. The mobile phase consisted of 0.05 M NaH_2PO_4 buffer pH 4.1–methanol (30:70, v/v) set at a flow rate of 1 mL/min at ambient temperature and UV detection at 247 nm.

Results The mobile phase consisting of 0.05 M NaH_2PO_4 buffer pH 4.1–methanol (30:70, v/v) set at a flow rate of 1 mL/min was selected. The retention time (RT) and asymmetry were found to be 4.042 ± 0.018 min and 1.05 ± 0.03, respectively (Figure 1). The constructed calibration curve was linear over the concentration range 16 ng mL^{-1} to 100 μg mL^{-1} with linear regression equation Y = 45351x + 4797.1 and regression co-efficient of 0.9997. The proposed method afforded recovery of 99.94–101.63% after spiking the additional standard drug concentration to the previously analysed test solution. The method was found to be robust as no significant change in the RT and %RSD were observed by changing the composition (4.027 ± 0.029 min, 1.436%), flow rate (4.074 ± 0.124 min, 1.18%) and pH (4.035 ± 0.0185, 0.458%) of the mobile phase. The assay result of Atorlip-10 tablet (Cipla Ltd., India) (n = 3) and nanoemulsion (Lab formulated) (n = 3) yielded 99.09% (% RSD 0.564%) and 100.16% (% RSD 0.53%), respectively.

Conclusions A simple, rapid, accurate, precise, selective and sensitive RP-HPLC analytical method has been developed and validated for the routine analysis of AT in tablets and nanoemulsion formulation with 99.09% and 100.16% recovery, respectively.

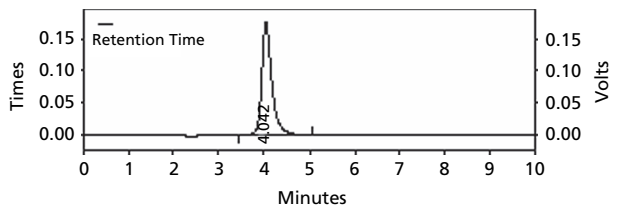

Figure 1 HPLC Chromatogram of atorvastatin.

Chaudhari, B. G., et al (2007) *Chem. Pharm. Bull*. **55**: 241–246
Shen, H. R., et al (2006) *Pharmazie* **61**: 18–20

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Development of a rapid and sensitive high performance liquid chromatographic method for determination of pioglitazone in human plasma

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Objectives Pioglitazone hydrochloride is an oral anti-hyperglycemic agent that acts primarily by increasing insulin sensitivity in target tissues. It is used both as monotherapy and in combination with sulfonylurea or insulin in the management of type 2 diabetes mellitus. In order to study the pharmacokinetics of pioglitazone in man, a rapid and sensitive assay for pioglitazone in biological fluids is necessary. Only a few liquid chromatographic methods have been reported in the literature to determine pioglitazone in biological fluids. However, these methods require time-consuming or complex sample preparation procedures and long chromatographic elution time for analysis of pioglitazone in plasma. Moreover, most of these methods need high percentage of organic solvents in mobile phase to obtain optimum separation. In this study a rapid, simple and sensitive High Performance Liquid Chromatographic method for determination of pioglitazone in plasma, using a monolithic stationary phase, has been developed.

Methods An analytical method based on High Performance Liquid Chromatography with ultraviolet detection (269 nm) was developed for the determination of pioglitazone in human plasma. Rosiglitazone was used as an internal standard. The chromatographic separation was performed on an analytical monolithic C18 column (100 × 3.9 mm, i.d) and a mobile phase consisting of acetonitrile-phosphate buffer (pH 3; 10 mM) (15:85) at a flow rate of 3.5 mL/min. For the plasma samples the protein was removed with acetonitrile.

Results Under the described chromatographic conditions, pioglitazone, rosiglitazone and endogenous plasma peaks were well-resolved in less than 5 min. Using UV detection at 269 nm, the detection limit for pioglitazone was 5 ng/mL. The calibration curve was linear over the concentration range 50–2000 ng/mL. The average recovery was 95% for plasma. The inter-day and intra-day assay coefficients of variation were found to be less than 8%.

Conclusion We have developed a rapid, simple, accurate and reproducible method for the determination of pioglitazone in plasma. This method will permit pharmacokinetic and pharmacodynamic studies of pioglitazone in man.

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Alternative method development for hazard reagents using method in Korean pharmaceutical codex

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Objectives The Korean pharmaceutical codex and ICH guideline recommend that hazardous reagents and solvents are restricted for human health and preservation of environment. But a few test methods using the hazard reagents (such as benzene, mercury compounds, cyanide compounds and dioxane) are now included in the monograph of the Korean pharmaceutical codex. Therefore to improve those cases in the Korean pharmaceutical codex, new alternative methods for 7 monographs will be developed and validated.

Methods The identification of safflower oil, pheniramine maleate, chlorphenamine maleate and L-cystine and the assay of bromopride, nicergoline and buflomedil were studied. In the identification test of thin-layer chromatography using benzene (class 1, Guideline for residual solvent by ICH) as mobile phase, it was substituted by heptane or methanol (class 2 or 3) in the new methods. The existing assay tests of bromopride and nicergoline were replaced by the potentiometric titration methods without using mercury compound. The existing assay method of buflomedil was replaced by HPLC method. The buflomedil was determined using a mixture of acetonitrile and potassium phosphate buffer pH 2.5 (65:35) as a mobile phase on Capcellpak C_{18} column and monitored at 280 nm.

Results New alternative methods were established without using hazardous reagents in 7 monographs. In the identification of safflower oil, pheniramine maleate, chlorphenamine maleate and L-cystine, we established the alternaitive methods to substitute benzene by heptane or methanol as mobile phase. In experiments, the spot from the test solution had the same Rf value as that of the standard solution and was well-defined in each detection limit concentration. New potentiometric titration methods of bromopride and nicergoline were established. An interlaboratory validation was also performed and the data were not significantly different ($P < 0.05$). To assay buflomedil, the HPLC method was developed and the precision, accuracy and linearity were validated. The correlation of standard solution showed the good results with 0.999 or above. It was also precise (RSD < 2.0%), accurate (recoveries is in 98.0–102.0%) and not affected by impurities.

Conclusions The 7 alternative methods were established in this study. This study could be useful for related experimenters in this field. Those developed methods will improve the specification of each monograph in the Korean pharmaceutical codex and be utilized for preparing revised version of the Korean pharmaceutical codex.

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Process for the development and validation of a near infrared spectroscopic whole tablet assay method

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Objectives To use near infrared spectroscopy (NIRS) to assay paroxetine 20 mg tablets, which contain 6.5%w/w of paroxetine. Conventional assay methods are time consuming and destructive. NIRS involves no sample preparation and a whole tablet can be assayed non-destructively in 30 s by relatively unskilled operators.

Methods A Foss 6500 near infrared spectrophotometer in transmission mode was used and tablets were presented to the instrument in an aluminium template.

The assay method was developed by collecting spectra with a range of potencies and producing a calibration against actual assay values determined by liquid chromatography. The range of potencies available using commercial batches was insufficient to develop a calibration and therefore small scale batches were produced using experimental design to produce tablets with varying potencies, hardness's, coating thicknesses and different excipients/drug substance batches. Multiple Linear Regression and Partial Least Squares were used to predict the assay values of a separate independent set of tablets.

Results The optimum method was found by taking 32 scans over a wavelength range of 850 nm–1300 nm. A second derivative spectral pre-treatment was used and 5 factor partial least squares was found to be the best mathematical correlation technique. The method was satisfactorily validated using the parameters below:

Specificity. A plot of the contributions of each wavelength to factor one was obtained. This resembled the second derivative spectrum of the drug substance showing evidence of specificity. Placebo tablets gave slightly negative assay results further showing specificity.

Linearity/range. A plot of the NIR predicted values versus HPLC reference values for the validation set gave a correlation coefficient of 0.98. The range was 15–23 mg/tablet.

Number of factors. The selection of the number of factors to choose was based on the minimum standard error in cross validation while not overfitting the data.

Standard error of prediction. The SEP was 0.46 mg/tab indicating no significant increase in errors on testing new samples.

System repeatability. The RSD of 6 assay results from one commercial tablet assayed six times was 0.64%.

Method repeatability. Five sets of ten tablets were assayed (RSD of the means was 0.21%).

Intermediate precision. One tablet from a commercial batch was presented six times to the instrument on two different days by two different analysts (RSD of the results was 0.58%).

Robustness. Robustness was built into the model by incorporating key variables within the manufacturing process and by incorporating commercial tablets batches selected over time. The alternative NIRS method could be run in parallel with the reference method (liquid chromatography), increasing the frequency of use of the alternative method with time. A control batch of tablets could be run on the system to verify ongoing method performance.

Conclusion NIRS allows a rapid non-destructive whole tablet assay and the possibility to test greater numbers of tablets. The technique lends itself to at line analysis where samples could be taken directly from a tablet press and assay values are checked by nearIR.

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Development of a gas chromatography method to identify *Levisticum officinale* in *Angelica sinensis* root

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Objectives The British Pharmacopoeia (BP) Commission is currently preparing a monograph for *Angelica sinensis* root for use in Traditional Herbal Medicine (Dang Gui). An important aspect of monographs for herbal medicines is that they should be able to distinguish the defined species from known adulterants or substituents or closely related species. A reported adulterant (Upton 2003) of *A. sinensis* is *Levisticum officinale* (lovage) and the aim of this study was to develop a method to identify the presence of this adulterant. Previously published methods using TLC (Upton 2003) and HPLC (Upton 2003; Lu et al 2005) have, due to the similarity between the chemical constituents in the two species, been unable to distinguish between the species (TLC) or have relied on simulated mean chromatograms and peak area ratios (HPLC). As an alternative, the use of gas chromatography (GC) to analyse volatile components isolated by hydrodistillation has been investigated in order to detect compounds that were present in *L. officinale* but absent in *A. sinensis*, and thus differentiate the two species.

Methods Samples of the herbal drugs were subjected to hydrodistillation for about 2 h until oily drops of volatile components were obtained. The oily drops were extracted using toluene and subjected to analysis by GC using an Agilent 6890N gas chromatograph; a capillary column (HP-5, 50 m × 0.32 mm i.d., film thickness 1.05 μm); helium as the carrier gas; 1 μL injection at 250°C with a split ratio of 1:20; oven temperature program of 40–220°C at 5°C/min then held at 220°C; FID detection at 250°C. MS detection using an Agilent MSD5973N with electron impact ionisation was also used to identify components using molecular weight comparisons and library searching.

Results The chromatograms obtained showed several peaks that were present in *L. officinale* but absent in *A. sinensis*. GC/MS analysis provided tentative identities of the various compounds and injection of standard solutions was used to confirm the identities. Four compounds were identified that were both present in *L. officinale* but absent in *A. sinensis* and readily available commercially. These were (–) carvone, octanoic (caprylic) acid, 3-propylidenephthalide and benzyl alcohol.

Conclusions GC analysis of its volatile components allowed positive identification of *L. officinale* in the presence of *A. sinensis*. This is important in the quality control of the popular traditional Chinese medicine Dang Gui. The inclusion of such a test in the BP monograph for *A. sinensis* root for use in Traditional Herbal Medicine will help to ensure that the correct herbal drug of an appropriate quality is available to patients in the UK. The method may also be useful to identify marker compounds for other adulterants of *A. sinensis*.

Lu, G.-H., et al (2005) *J. Chromatogr. A* **1073**: 383–392
Upton, R. (ed.) (2003) *American herbal pharmacopoeia*. Dang Gui Root, 1–41

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Investigation of universal standard materials for the transfer of quantitative calibrations in near-infrared spectroscopy

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Objectives The aims of this study were to explore the use of universal standard materials for near-infrared (NIR) quantitative calibration transfer from one instrument to another rather than using real samples of pharmaceutical products.

Methods Lipitor core tablets (180 tablets), containing 7.5–12.5 mg of atorvastatin (Pfizer, Sandwich) were used for developing calibration models. Reference analytical data for the tablets were provided by Pfizer. The model was transferred between two similar spectrometers: a Foss NIRSystem XDS spectrometer with a Rapid Content Analyzer (instrument A) and Foss NIRSystem 6500 monochromator with a Rapid Content Analyzer (instrument B) located in different laboratories. Different materials were investigated with 11 demonstrating good spectral properties. These were studied for reproducibility among different batches and suppliers. These materials were prepared in three ways: grinding, sieving and pressing to minimise variation of the batches within the same material. To study the effect of sample diameter on the spectrometer, sucrose and microcrystalline cellulose PH101 (MCC PH101) were filled in two containers with different diameters. The calibration models were developed on instrument A, and then transferred to instrument B using various correction methods and using the spectra of the product and the standard materials.

Results The sieved samples showed the highest reproducibility with the highest correlation coefficient (> 0.999) between paired spectra of samples of the same material. Therefore, this approach was selected to prepare the samples for transfer standards. The spectra from instrument A were treated by SNV following 1st derivative SG filter (71 points, second-order polynomial). Then, calibration models were generated by PLS regression models and the best model was one with 10 factors utilising the spectral range 1150.5–1447 nm, 1580.5–1745 nm and 1965–2412 nm. This model gave RMSEC = 0.079% m/m and RMSEP = 0.080% m/m with bias –0.019% m/m. The coefficient of multiple determination (R^2) of calibration was 0.992 and of validation was 0.991. Moreover, the model showed no statistical difference at the 5% significant level ($P = 0.059$) for paired Student's t-test between the validation and reference data and showed no significant differences between slope and intercept from 1 and 0, respectively. Overall, the calibration model was fit for purpose. The model was transferred to instrument B by direct transfer (RMSEP = 0.868% m/m) and spectral correction using different standards: mean sample residual spectrum (RMSEP = 0.168% m/m), MCC PH101 filled in Foss cell (RMSEP = 0.244% m/m), MCC PH101 filled in Waters 4 mL vials (RMSEP = 0.351% m/m) and sieved sucrose filled in Waters 4 mL vials (RMSEP = 0.520% m/m).

Conclusions This study shows that universal standard materials are not as good as using the mean sample residual spectrum to transfer quantitative calibrations from one NIR instrument to another. The similarity of the transfer samples to the real sample population (chemical composition and physical dimensions) is the most important factor to consider in calibration transfer.

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High performance liquid chromatography assay method for simultaneous quantitation of formoterol and the two epimers of budesonide

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Objective The aim of this study was to develop a sensitive and a simultaneous HPLC method for the analysis of formoterol and the two epimers of budesonide

hence it is required by European pharmacopoeia to keep a fixed ratio between the two epimers.

Method This method used a Capital ODS2 Spherisorb 5 micrometer 250 mm 4.6 mm i.d. with a mobile phase consisting of acetonitrile phosphate buffer (pH 3.0; 7.5 mM) (40:60, v:v) and a flow rate of 1.0 mL/min; two wave lengths were used to analyse the pharmaceutical preparations, the first wave length was 214 nm for formoterol and the second was 240 nm for budesonide.

Result Validation studies demonstrated that the method possessed a linear UV response, high system precision and accuracy, high sensitivity and specificity for formatrol and two epimers of budesonide. The limit of detection (LOD) and the limit of quantitation (LOQ) for formoterol assay method were 5.401 mcg/L and 18.003 mcg/L respectively. In addition, the LOD and LOQ for budesonide A assay method were 134.525 mcg/L and 448.416 mcg/L, respectively. Finally, The LOD and LOQ for Budesonide B assay method were 62.27 mcg/L and 207.566 mcg/L, respectively.

Conclusion An isocratic liquid chromatographic method has been described, optimised and validated for simultaneous qualitative and quantitative determination of formoterol fumarate and budesonide epimers. Acceptable assay precision and accuracy and excellent linearity was achieved. In addition to its high sensitivity and robustness, the proposed HPLC method proved reliable determination of the budesonide and formoterol delivered from the Symbicort Turbuhaler. As result this method can substitute the two separate methods, and the single method for budesonide and formoterol since it can determine both budesonide epimers. For that reason, this method will save both cost and time.

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Sensitive and simple liquid chromatography-tandem mass spectrometric (LC-MS/MS) method for estimation of valsartan in human plasma

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Objective Valsartan is an orally active nonpeptide specific angiotensin II type I receptor blocker effective in lowering blood pressure in hypertensive patients. There are few methods reported in the literature for estimation of valsartan in plasma, most of them being HPLC with UV or fluorescence detection and seldom LC-MS/MS. The objective this study was to develop a simple, rapid and sensitive LC-MS/MS method for the estimation of valsartan in human plasma that can be utilized for assessment of pharmacokinetics/bioavailability/bioequivalence of valsartan formulations.

Table 1 Validation parameters for the proposed LC-MS/MS method for estimation of valsartan in human plasma

Validation parameter	Results (valsartan)
Sensitivity (ng/mL) LOQ	25.205
Linearity range (ng/mL)	25.205–5041.0
Accuracy (%)	
Within-batch	92.12–103.53
Between-batch	96.43–101.83
Ruggedness (different analyst)	94.52–103.53
Precision (%CV)	
Within-batch	0.38–5.17
Between-batch	1.94–6.23
Ruggedness (different analyst)	1.73–3.09
Recovery (%)	92.11 (89.05 – ISTD)
Dilution integrity (accuracy %)	
(1:2 dilution)	103.90
(1:4 dilution)	94.78
Stock solution stability	
Short-term (7.0 h)	100.67% (100.35% – ISTD)
Long-term (16 days)	99.42% (101.32% – ISTD)
Plasma sample stability	
Freeze-thaw (3 cycles)	104.86% (LQC); 103.10% (HQC)
Bench-top (6.0 h)	99.80% (LQC); 97.99% (HQC)
Autosampler (25.5 h)	100.06% (LQC); 103.21% (HQC)
Long-term (29 days)	109.09% (LQC); 111.81% (HQC)

Methods Valsartan and the internal standard (ISTD), losartan, were extracted from human plasma (200 μL) using methanol and the supernatant was directly injected onto the LC-MS/MS system (Sciex API4000 LC-MS/MS with electrospray ionization (ESI) turboionspray inlet). The chromatographic conditions were as follows: column – Kromasil 100 C_{18} (5 μ, 250 × 4.6 mm); mobile phase – mixture of ammonium acetate buffer (25 mM, pH 4.50 ± 0.05)–methanol (25:75 v/v); flow rate – 1 mL/min. Valsartan and ISTD were monitored in the positive ion-multiple reaction monitoring (MRM) mode at transitions of 436.5→235.3 (m/z) and 423.3→207.1 (m/z), respectively.

Results The method employed a simple and single step extraction, enabling faster sample processing. Elution of valsartan and ISTD was achieved in 4 min providing rapid analysis. The method is specific and sensitive requiring only 200 μL of sample with limit of quantification of 25.205 ng/mL. The method was validated and the validation parameters are summarized in Table 1.

Conclusion The present LC-MS/MS method offers a number of advantages, such as shorter analysis time, smaller sample volume (200 μL blood) and simple sample cleanup. The method was found to be simple, rapid, sensitive, precise, accurate and specific for estimation of valsartan in human plasma and was successfully employed for bioavailability/bioequivalence studies of valsartan.

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A sound-based strategy to assist in data interpretation

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Objectives As modern analytical techniques become more elaborate, several 'layers' of data may be acquired during a single experiment. This means that the analyst is left with options of ignoring large parts of the data set, or spending considerable time and effort sifting through multidimensional data. Methodologies to assist scientists in the interpretation and analysis of this multi-dimensional data fall into two overall approaches: data filtration and improved human computer interface. The work presented here focused upon sonification as a strategy to improve the human computer interface for large dimensional datasets produced by separation-based analytical techniques. Sonification is defined as the use of 'non-speech audio' to represent data and has been used successfully in other areas of science.

Methods In this study we designed a Java based interface for presenting sonified chromatograms and conducted a volunteer study (n = 13) among practicing chromatographers to assess the sonification strategy. The volunteers were given tasks to perform using three interfaces: a visual-only mode (similar to that used in modern chromatography data systems), a sonification-only mode and a combined visual-sonification mode. Before the study, volunteers were asked to classify themselves in terms of their chromatographic and musical experience. After the tasks had been performed users rated the various interfaces via a NASA TLX analysis grid. The study was performed according to the University of Strathclyde's Code of Practice on Investigations on Human Beings.

Results The accuracy for the visual, sonification and combined interfaces were equivalent at 99%, 98% and 99%, respectively. The overall times to complete the tasks for the visual, sonification and combined interfaces were 288 s, 258 s and 207 s, respectively, but the *t*-test between the visual and sonification completion times was not statistically significant. A small set of users who rated themselves as having a strong musical ability performed the tasks faster in the sonification mode than the visual. Users who rated themselves as having musical ability reported a preference for the sonification interface over the normal visual interface.

Conclusions This study succeeded in testing a sonification interface for normal analysis-based tasks on practicing chromatographers. The results demonstrate that sonification strategies can effectively convey experimental results to chromatographers.

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Validation of HPLC method for the quantification of tobramycin in urine samples after inhalation using pre-column derivatisation with fluorescein isothiocyanate

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Objective A reversed-phase liquid chromatography method involving pre-column derivatisation with fluorescein isothiocyanate (FITC, isomer I) for determination of tobramycin in urine samples after inhalation has been developed. FITC reacts with the primary amino groups of tobramycin and other aminoglycosides under mild conditions to form a highly fluorescent and stable derivative.

Method The chromatographic separation was carried out on a Phenomenex Luna C_{18} column at ambient temperature using a constant flow rate of 1 mL/min and mobile phase of acetonitrile–methanol–glacial acetic acid–water (420:60:5:515, v/v/v/v). The tobramycin-FITC derivative was monitored by fluorescent detection at an excitation wavelength 490 nm and emission 518 nm.

Results The linearity of response for tobramycin was demonstrated at eleven different concentrations of tobramycin extracted from spiked urine, ranging from 0.25 to 20 μg/mL. Tobramycin and neomycin were extracted from spiked urine by a solid phase extraction clean-up procedure on a carboxypropyl-bonded phase (CBA) weak cation-exchange cartridge, and the relative recovery was >99% (n = 5). The limit of detection (LOD) and limit of quantitation (LOQ) in urine were 70 and 250 ng/mL, respectively. The method had an accuracy of <0.2%, and intra-day and inter-day precision (in term of % coefficient of variation) were < 4.89% and 8.25%, respectively.

Conclusion The method reported is simple, reliable, precise and accurate and has the capability of being used for urinary pharmacokinetic studies to identify the relative lung deposition of tobramycin after inhalation of tobramycin inhaled solution 300 mg/5 mL (TOBI) by different nebulizer systems.