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The use of 96-well Scintiplates to facilitate definitive metabolism studies for drug candidates

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Abstract

Semi-quantitative analysis of the drug-related components in biological samples collected during definitive metabolism studies using radiolabelled drug candidates is commonly achieved by HPLC profiling, using either on-line radiochemical detection or off-line liquid scintillation counting (LSC) following collection of the HPLC eluent into vials. However, although the use of LSC with vials has high sensitivity, the approach is time-consuming, laborious and destructive, whilst on-line detection methods are inappropriate for samples with low-levels of radioactivity (commonly the case with plasma samples). The use of 96-well microtitre plates (Scintiplates) for fraction collection during HPLC profiling provides a sensitive, effective and efficient alternative method for the semi-quantitative analysis of radiolabelled components in biological samples. Furthermore, the approach is non-destructive, such that subsequent identification of the isolated components can be achieved. Although the Scintiplate methodology is not appropriate for the analysis of excreta samples, where quenching of the radiochemical signal by endogenous components was observed, the approach was demonstrated to be valid for the relative quantification of [^{14}C]-labelled material in plasma samples for all species investigated. In addition, good sensitivity was observed, with a counting efficiency of 79% for [^{14}C], such that a drug-related component accounting for 10–15 dpm is quantifiable. The utility of the methodology for profiling circulating metabolites was demonstrated by the analysis of a rat plasma sample following oral administration of [^{14}C]-UK-349,862. The Scintiplate approach and subsequent mass spectrometric analysis resulted in the relative quantitation and specific characterisation of circulating metabolites accounting for 93% of the total plasma radioactivity.

Keywords: 96-Well Scintiplates; Radiochemical detection; Metabolite profiling; Metabolite identification; Mass spectrometry

1. Introduction

The identification and relative quantitation of metabolites is fundamental to underwrite the safety profile of novel drugs [1,2]. Conventional practice is to carry out definitive metabolism investigations for all development candidates during radiolabelled studies in appropriate animal species and in man, typically using either [^{14}C] or [^{3}H] isotopes. With the aim of specific characterisation of all major excreted and circulating metabolites, together with their relative quantitation as a percentage of the total dose (for excreted components) or a percentage

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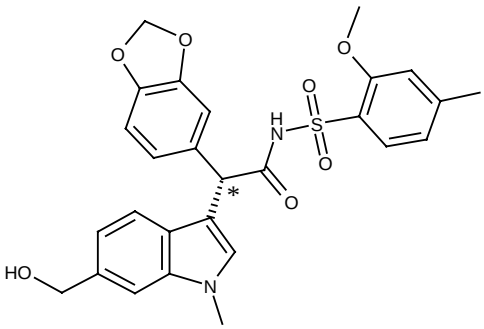
of the total circulating radioactivity (for circulating metabolites), methods for the structural elucidation and the measurement of the radiochemical response of each component are critical. The most common method used to monitor drug-related material during HPLC profiling is on-line radiochemical flow detection, using either solid cells, packed with a solid scintillator (heterogeneous detection), or liquid cells, where liquid scintillant is added to the HPLC eluent as it passes through the cell (homogenous detection) [3–8]. The on-line approach is attractive, due to its rapid throughput and, in the case of heterogeneous detection, its non-destructive nature, such that identification and relative quantitation of the drug-related components can be achieved in the same analysis. The disadvantage, however, is poor sensitivity, due to the relatively low residence time of the radioactive components in the cell at conventional HPLC flow-rates. Although the use of homogenous on-line detection gives improved sensitivity, particularly for [^{3}H]-labelled material, signal-to-noise remains a significant problem when the levels of radioactivity are low. Much improved counting efficiency can be achieved by fraction collection into vials during HPLC analysis and off-line scintillation counting following the addition of liquid scintillant to the isolated samples. In this way, the counting time can be increased, thus dramatically lowering the limit of detection. However, collection into vials is a destructive approach and is also extremely time-consuming and laborious. For higher throughput, fractions have been collected into 96-well microtitre plates with a solid scintillant coated onto the surface of the wells [9,10]. The plates can therefore be dried and counted without the addition of liquid scintillant. However, as a result of the fragile nature of the scintillant bed within the well, subsequent analysis of the isolated components by mass spectrometry is not possible. The work presented here will describe the use of a different type of 96-well microtitre plate (Scintiplates) where the scintillant is embedded in the polystyrene matrix of the wells. Using these plates, the fraction collection approach becomes non-destructive, enabling subsequent structural elucidation of the drug-related components after relative quantitation has been achieved. Data are presented to demonstrate the use of 96-well Scintiplates both to generate radiochemical profiles of good signal-to-noise and chromatographic resolution, and also enable the subsequent mass spectrometric identification of the drug-related components. In this way, metabolite detection, resolution, relative quantitation and identification can be achieved from a single analysis.

2. Experimental

2.1. Chemicals

UK-349,862 ([(*S*)-(+)-3-{1-(1,3-benzodioxol-5-yl)-2-[(2-methoxy-4-methylphenyl)sulfonamido]-2-oxoethyl}-6-(hydroxymethyl)-1-methyl-1*H*-indole]) was synthesised at Pfizer Global Research and Development, Sandwich, UK. [^{14}C]-UK-349,862 (Fig. 1) was prepared by Amersham Biosciences UK (Cardiff, UK) with a radiochemical purity of 99% by HPLC and a specific activity of 37.4 μCi/mg. Two Pfizer development compounds, one labelled with [^{14}C] (specific activity: 47.3 μCi/mg; radiochemical purity: 98.6% by HPLC) and one with [^{3}H] (specific activity: 5 Ci/mmol; radiochemical purity: 98.9% by HPLC), were used as test compounds for the validation work and counting efficiency assessment. The structures cannot be revealed for proprietary reasons. Glacial acetic acid, tris(hydroxymethyl)aminomethane (Tris Base) and tris(hydroxymethyl)aminomethane hydrochloride (Tris–HCl) were purchased from Fisher Scientific Co. (Loughborough, Leics, UK). Ammonium acetate, sodium hydroxide, hydrochloric acid (0.1 M) and HPLC grade methanol were purchased from Sigma–Aldrich (Gillingham, Dorset, UK). Water



* denotes position of [^{14}C] label

Fig. 1. Structure of [^{14}C]-UK-349,862.

was purified using a Milli-Q system (Millipore Corporation, Molshein, France).

2.2. Preparation of 96-well Scintiplates for method validation

96-Well Scintiplates (Perkin-Elmer Life Sciences, Boston, MA) were filled with a methanolic solution (100 μl per well) of one of the radiolabelled test compounds using a 12-tip pipette (Eppendorf, Hamburg, Germany). Varying concentrations of radioactivity were used, corresponding to approximately 40, 400 or 4000 dpm per well for each isotope. The plates were dried in a DD4 vacuum centrifuge (Genevac Ltd., Ipswich, Suffolk, UK), maintaining the rotor and chamber temperature at ≤30 °C, and then analysed for total radioactivity using a six-detector Microbeta 1450 scintillation counter (Perkin-Elmer Life Sciences) with a counting time of 5 min per well.

2.3. Preparation of biological samples from undosed subjects for method validation

Rat, dog and human faecal homogenates were prepared by mixing faecal samples with water (1:1, w/w). Aliquots of each homogenate (4 g) were mixed with methanol (25 ml) followed by sonication. In each case, the mixture was centrifuged at 3500 rpm (1643 × *g*) for 5 min using a CR312 instrument (Jouan, St. Nazaire, France) and the supernatant was collected. The extraction procedure was repeated using a mixture of methanol (24 ml) and Tris buffer (pH 6; 0.1 M; 1 ml) and a mixture of methanol (23 ml) and Tris buffer (pH 9; 0.1 M; 2 ml). The combined extracts were reduced to dryness under nitrogen in a Turbovap (Zymark Corp., Hopkinton, MA, USA) at 37 °C and the residue was reconstituted in ammonium acetate (pH 5; 0.1 M; 2 ml) for HPLC analysis.

Rat, dog and human urine (2 ml) was centrifuged at 3500 rpm (1643 × *g*) for 15 min using a CR312 instrument (Jouan) prior to HPLC analysis.

Samples of rat, dog and human plasma (1 or 5 ml) were treated with methanol (5 or 10 ml, respectively), mixed and centrifuged at 3500 rpm (1643 × *g*) and 4 °C for 15 min using a CR312 instrument (Jouan). In each case, the supernatant was collected and reduced to dryness under nitrogen in a Turbovap (Zymark) at 37 °C and the residue was reconstituted in ammonium acetate (pH 5; 0.1 M; 2 ml) for HPLC analysis.

2.4. HPLC profiling of biological samples for the determination of quenching effects

The prepared excreta and plasma samples were profiled by HPLC using a HIRPB column (25 cm × 7.75 mm) (Hichrom Ltd., Reading, Berkshire, UK) at a flow-rate of 2 ml/min with a 0–100% methanol-ammonium acetate (pH 5; 0.1 M) binary solvent gradient over 30 min, followed by 100% methanol for 5 min, returning to the initial conditions over 5 min. The HPLC system involved two LC-10AS pumps (Shimadzu Corp., Kyoto, Japan) and a SCL-10A system controller (Shimadzu). For each species (rat, dog and human), faecal extracts (corresponding to 0.4, 1 and 2 g of faecal homogenate), urine (100, 500 and 1000 μl) and plasma following protein precipitation (derived from 1 and 5 ml plasma) were analysed. Fractions (8.4 s) were collected using a 222XL liquid handler (Gilson Inc., Villers le Bel, France) on to 96-well Scintiplates (Perkin-Elmer Life Sciences), which had been previously applied with one of the radiolabelled test compounds. The plates were dried in a DD4 vacuum centrifuge (Genevac), maintaining the rotor and chamber temperature at ≤30 °C, and then analysed for total radioactivity using a six-detector Microbeta 1450 scintillation counter (Perkin-Elmer Life Sciences) with a counting time of 5 min per well.

2.5. Determination of the counting efficiency of the Scintiplate approach

Replicate ($n = 6$) aliquots (100 μl) of radioactive solutions (containing one of the radiolabelled test compounds) at varying concentrations (200, 2000 and 20000 dpm per well) were applied to a 96-well Scintiplate (Perkin-Elmer Life Sciences). The plates were dried in a DD4 vacuum centrifuge (Genevac), maintaining the rotor and chamber temperature at ≤30 °C, and then analysed for total radioactivity using a six-detector Microbeta 1450 scintillation counter (Perkin-Elmer Life Sciences) with a counting time of 5 min per well.

2.6. Absorption, distribution, metabolism and excretion (ADME) study in rats

Female Sprague–Dawley rats (~250 g; $n = 4$; Charles River, Manston, Kent, UK) received a single oral dose of [^{14}C]-UK-349,862 (25 mg/kg; nominally 50 μCi) dissolved in 0.1 M sodium hydroxide adjusted to pH 9 with 0.1 M hydrochloric acid. Doses were administered by gavage at a dose volume of 8 ml/kg. Blood samples (approximately 5–10 ml) were collected by terminal exsanguinations under isofluorane anaesthesia into heparinised tubes 4 h post-dose. Plasma was prepared by centrifugation and stored frozen prior to analysis.

2.7. HPLC analysis of rat plasma following oral (25 mg/kg) administration of [^{14}C]-UK-349,862

Female rat plasma (4 h post-dose; 70 μl) following single oral (25 mg/kg) administration of [^{14}C]-UK-349,862 was treated with methanol (1 ml) and the sample was centrifuged at 3500 rpm (1643 × *g*) and 4 °C for 15 min using a CR312 instrument (Jouan). The supernatant was collected and reduced to dryness under nitrogen on a Turbovap (Zymark) at 37 °C. The dried sample was reconstituted in methanol-ammonium acetate (pH 5; 0.1 M) (40:60, v/v; 1 ml) and an aliquot (equivalent to 5000 dpm total radioactivity) was profiled by HPLC using a HIRPB column (25 cm × 7.75 mm) (Hichrom) at a flow-rate of 2 ml/min and a 40–70% methanol-ammonium acetate (pH 5; 0.1 M) binary solvent gradient over 30 min, followed by 100% methanol for 5 min, returning to the initial conditions over 5 min. Fractions (8.4 s) were collected into 96-well Scintiplates (Perkin-Elmer Life Sciences), which were dried in a DD4 vacuum centrifuge (Genevac), maintaining the rotor and chamber temperature at ≤30 °C, and then analysed for total radioactivity using a six-detector Microbeta 1450 scintillation counter (Perkin-Elmer Life Sciences) with a counting time of 5 min per well. A further aliquot of precipitated plasma (equivalent to 5000 dpm total radioactivity) was reconstituted and profiled using the same HPLC conditions, collecting fractions (15 s) into 7 ml scintillation vials (Meridian, Surrey, UK), to which were added Starscint scintillation cocktail (2 ml; Packard Bioscience Company, Meriden, CT, USA) prior to analysis for total radioactivity on a Guardian liquid scintillation counter (Perkin-Elmer Life Sciences) with a counting time of 5 min per sample.

2.8. Mass spectrometric identification of the circulating metabolites of [^{14}C]-UK-349,862

Mass spectrometric analysis of the circulating metabolites of [^{14}C]-UK-349,862 was carried out on an API4000 mass spectrometer (MDS Sciex, Concord, ON, Canada) in positive TurboIonSpray mode. The mass spectrometric conditions were optimized by variation of the declustering potential, collision energy and TurboIonSpray temperature. Nitrogen was used as the collision, TurboIonSpray, nebuliser and curtain gas. The resolution was set at 0.7 atomic mass units (amu) width at half-height in both Q1 and Q3. The drug-related components were eluted from the 96-well Scintiplates (Perkin-Elmer Life Sciences) using methanol-water (both containing ammonium acetate (2 mM)) (70:30; 200 μl) and infused directly into the mass spectrometer at a flow-rate of 5 μl/min using a model 22 infusion pump (Harvard Apparatus, SouthNatick, MA, USA) following appropriate dilution.

3. Results and discussion

3.1. Validation of the Scintiplate approach

Fraction collection into scintillation vials during HPLC profiling followed by the addition of liquid scintillant for off-line counting is a laborious and time-consuming process. However, the approach does have the potential to incorporate external standardisation, thus correcting for chemical and colour quenching of the radiochemical signal by sample components. In contrast, collection of fractions into 96-well Scintiplates followed by scintillation counting of the dried plates on a Microbeta instrument incorporates no quench correction. As HPLC profiling of ADME study samples will often involve significant quantities of crude biological matrices, it was important to evaluate the potential quenching effects of endogenous matrix components on the radiochemical response using the Scintiplate approach. To achieve

this, plates were spiked with a known amount of radioactivity (approximately 4000 dpm per well), dried and counted. The plates were then used to collect the HPLC eluent during the analysis of varying amounts of the biological matrices typically used in drug development metabolism studies (faecal extracts, urine and plasma following protein precipitation). To evaluate inter-species variation, samples from the three most common species used in development metabolism studies, rat, dog and human, were used for the analysis. Following HPLC profiling of the biological samples, the plates were dried again and re-counted, such that the difference between the two sets of counting data would reflect the degree of quenching caused by the endogenous components in the matrices under investigation. In all cases, the amounts of biological matrix were chosen to cover the typical range used in conventional drug development metabolism studies. As the majority of radiolabelled studies in these laboratories are conducted with [^{14}C]-labelled material, the validation work was focused on this isotope. However, as [^{3}H]-labelling is occasionally used, and [^{3}H] is likely to be more susceptible to quenching effects due to the lower energy of the beta particles, the effect of endogenous material on the [^{3}H] signal was evaluated for plasma samples. For both isotopes, little difference in quenching effect was observed between the three species under investigation. Therefore, data are only presented for rat samples to exemplify the results of the validation.

As expected, the degree of quenching of the radiochemical signal by faecal homogenate extracts was high in all species investigated. Even following the injection of relatively small amounts of material (equivalent to 0.4 g faecal homogenate), quenching was considerable (Fig. 2a), whilst for larger quantities

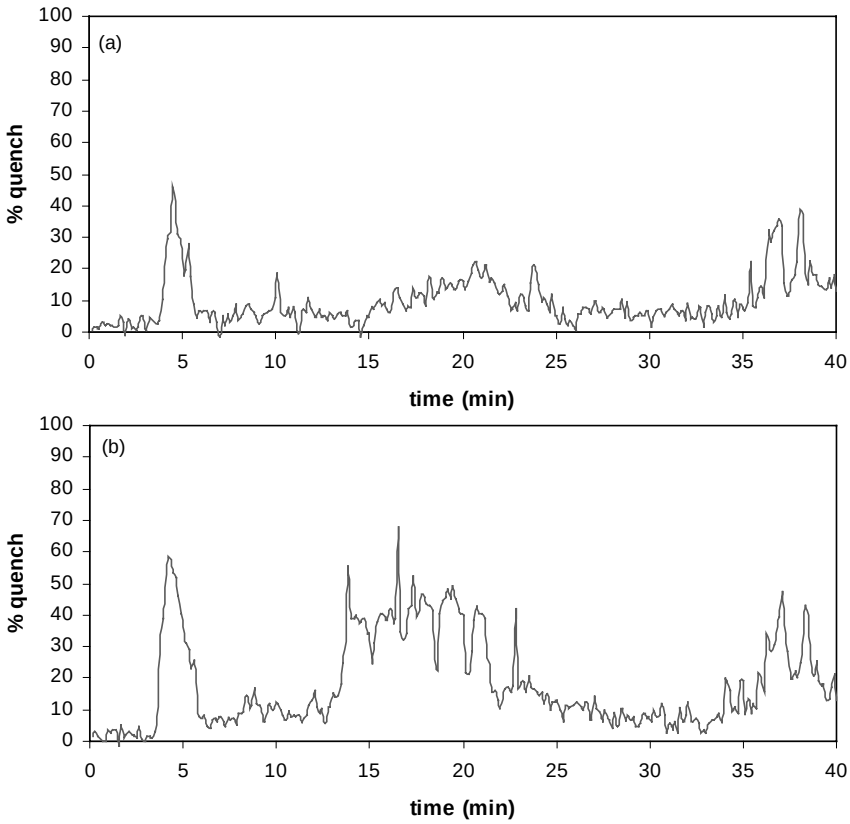


Fig. 2. Quenching effect of rat faecal extract equivalent to (a) 0.4 g faecal homogenate and (b) 1 g faecal homogenate on the [^{14}C] radiochemical signal using the Scintiplate approach.

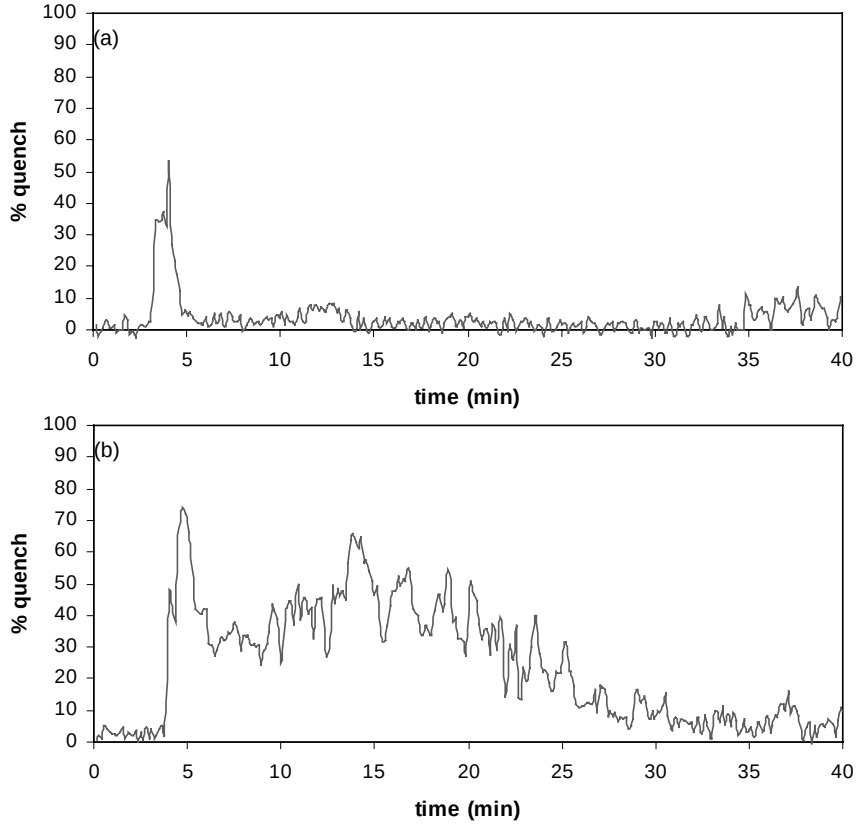


Fig. 3. Quenching effect of (a) 100 μl and (b) 1 ml rat urine on the [^{14}C] radiochemical signal using the Scintiplate approach.

of faecal extract, the effect was dramatic (Fig. 2b). For urine, the degree of quenching was less marked than for faecal extracts. At low injection volumes (100 μl), little significant quenching was observed for [^{14}C] in all species other than at the solvent front, where the high concentration of polar endogenous material gave a marked effect (Fig. 3a). At injection volumes that may be more typical of conventional studies (>500 μl), however, quenching of the radiochemical signal was large for all species under investigation (Fig. 3b). For excreta samples, therefore, it is clear that endogenous matrix components result in significant quenching of the radiochemical signal. Furthermore, the effect is not constant over a typical HPLC solvent gradient, and therefore will potentially result in invalid relative quantitation data. As the composition of faecal homogenate samples and the dilution of urine samples will vary significantly, it is possible that the Scintiplate approach may be applicable to excreta profiling in some cases. Furthermore, additional pre-treatment of faecal or urine samples might alleviate the quenching effect to some extent. Nevertheless, it is unlikely that the use of Scintiplates will give a valid measure of radiochemical response during excreta profiling unless the levels of radioactivity are very high and therefore the effect of the endogenous components can be reduced by dilution. Even in cases such as this, however, care should be taken to evaluate the quenching effect of the matrix components, for example by comparison of the radiochemical profile using Scintiplates with the equivalent data generated by fraction collection into scintillation vials followed by off-line liquid scintillation counting (LSC).

In contrast to excreta profiling, only low levels of quenching of the [^{14}C] signal were observed following protein precipitation and HPLC profiling of plasma from all species under investigation, even when samples derived from large volumes of plasma (5 ml) were

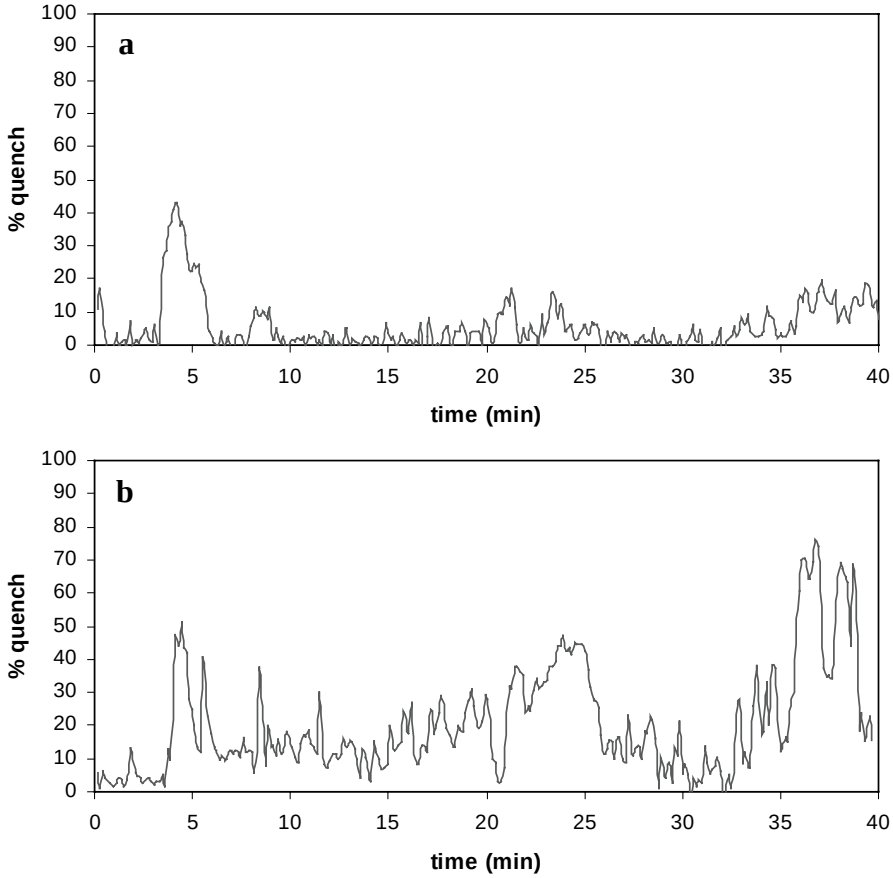


Fig. 4. Quenching effect of rat plasma (5 ml) following protein precipitation on (a) the [^{14}C] radiochemical signal and (b) the [^{3}H] radiochemical signal using the Scintiplate approach.

used (Fig. 4a). Only at the solvent front, where the levels of residual endogenous components following protein precipitation are relatively high, does significant quenching occur for [^{14}C]-labelled material. For this reason, the possibility of affecting the relative quantitation data is small. For [^{3}H]-labelled material, however, quenching of the radiochemical signal by plasma components was large for all species (Fig. 4b), due to the lower energy of the beta particles.

In order to assess whether the low degree of quenching of [^{14}C]-labelled material by plasma components was affected by the ratio of radioactive to endogenous material, further validation work was carried out with precipitated plasma samples involving varying amounts of applied radioactivity. Values ranging from 40 to 4000 dpm per well were chosen in order to determine whether low-level radioactive plasma samples might be profiled by the Scintiplate approach without significant quenching of the [^{14}C] radiochemical signal. The data showed that quenching caused by large volume (5 ml) plasma samples was low for all species at all levels of applied [^{14}C] radioactivity. It would appear, therefore, that for profiling circulating [^{14}C]-labelled metabolites, the Scintiplate approach will give an accurate representation of the radiochemical response and thus provide valid relative quantitation data. In contrast to excreta samples, the levels of drug-related material in plasma are often too low to enable on-line radiochemical detection, such that the Scintiplate approach could represent a new and improved method for profiling circulating metabolites.

3.2. Counting efficiency of the Scintiplate approach

A known amount of radioactivity ([^{14}C] or [^{3}H]) was added to a 96-well Scintiplate, which was then dried and counted on a Microbeta scintillation counter. In order to assess the effect of the absolute radioactivity level on the counting efficiency, replicates ($n = 6$) at three different radioactivity levels were applied to the plate for each isotope. Using the mean counting data ($n = 18$), the efficiency for [^{14}C] was determined as 79%. Although not as high as liquid scintillation counting (typically 90–95%), this efficiency is likely to yield acceptable counting data in most cases. Little variation in efficiency for [^{14}C] with the absolute level of radioactivity was observed; over the 100-fold range used in this study, the standard deviation and % coefficient of variation (% CV) of the efficiency data were 1.5 and 2%, respectively. The measured efficiency value was used to determine the limit of detection (LOD) of the Scintiplate approach. Using a counting time of 5 min per well, the calculated LOD for [^{14}C] was approximately 7 dpm per well [11]. In principle, therefore, a drug-related component accounting for

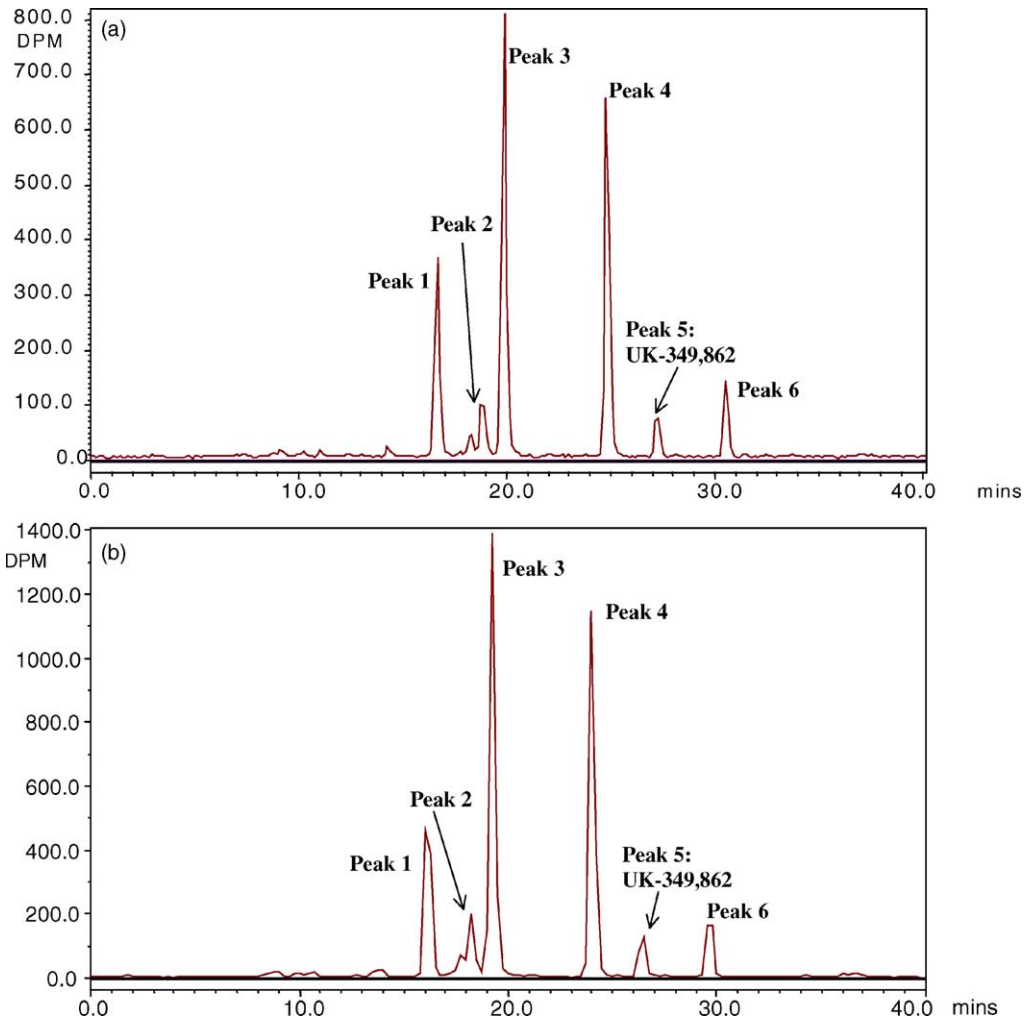


Fig. 5. Reconstructed radiochromatogram for female rat plasma (4 h) following oral administration of [^{14}C]-UK-349,862 collecting fractions into (a) 96-well Scintiplates and (b) scintillation vials.

7 dpm should be quantifiable using the approach. However, such a calculation assumes that the component is collected in a single well, which is highly unlikely if fraction times are short and therefore good chromatographic resolution is to be achieved. Realistically, therefore, with typical fraction sizes and flow-rates, the LOD will be higher than the calculated value. Nevertheless, an LOD of approximately 10–15 dpm is certainly achievable. As with all radiochemical profiling, chromatographic resolution and absolute sensitivity must be balanced on a case-by-case basis. As expected, the lower energy of the beta particles emitted from [^{3}H]-labelled material resulted in a lower counting efficiency of 17% ($n = 18$) for this isotope. This value compared with approximately 45–50% efficiency using liquid scintillation counting. Furthermore, the efficiency results were more variable than for [^{14}C], with a standard deviation of 2.3 and a % CV of 13.4. These data give a theoretical LOD of approximately 16 dpm per well for [^{3}H].

3.3. Metabolite profiling of rat plasma following oral administration of [^{14}C]-UK-349,862

UK-349,862 is a potent endothelin A receptor antagonist developed as a potential treatment for conditions such as hypertension and chronic heart failure [12–14]. Following protein precipitation with methanol, female rat plasma following oral administration of [^{14}C]-UK-349,862 was profiled by HPLC, collecting fractions into 96-well Scintiplates. After an injection of approximately 5000 dpm, off-line counting of the dried plates resulted in a reconstructed radiochemical profile of good signal to noise (Fig. 5a). In order to assess the validity of the relative quantitation data, a further sample of precipitated plasma was profiled, collecting fractions into vials for off-line counting following the addition of liquid scintillant. The resulting profile (Fig. 5b) was very similar to the radiochromatogram generated using Scintiplates, such that the relative quantitation data for the six major drug-related components in terms of the percentage total plasma radioactivity were essentially identical for the two methods (Table 1). The six major drug-related regions were eluted from the Scintiplates with a small volume of a solvent amenable to mass spectrometric analysis and infused directly into the mass spectrometer following appropriate dilution. By using suitable precursor and neutral loss experiments based on the MS/MS fragmentation of the parent compound, all the major circulating components were detected and subsequently identified on the basis of their MS/MS data. The major metabolites were identified as a glucuronide conjugate of parent compound (Peak 3), accounting for 33% of the total plasma radioactivity and a carboxylic acid metabolite (Peak 4) resulting from oxidation of the primary alcohol functionality (28%). Unchanged UK-349,862 (Peak 5), an aldehyde intermediate in the oxidation of the alcohol to the carboxylic acid (Peak 6) and a diacid resulting from hydrolysis of the carbamide moiety (Peak 1) were identified as minor components. In addition, an acyl glucuronide conjugate of the carboxylic acid resulting from oxidation of the primary alcohol functionality was identified in the plasma sample (Peak 2). Although the drug-related region containing Peak 2 clearly comprised more than one radiolabelled component, no evidence was observed for any additional drug-related compounds. For this reason, it is possible to speculate that acyl migration of the acyl glucuronide conjugate to form two or more isomeric conjugates may be occurring [15–17]. The final metabolic scheme for female rat plasma following oral administration of [^{14}C]-UK-349,862 is shown in Fig. 6. The data show how the Scintiplate approach was used to achieve the resolution, relative quantitation and specific characterisation of drug-related components accounting for 93% of the total plasma radioactivity.

Table 1
Comparison of relative quantitation data derived from fraction collection into Scintiplates and scintillation vials for the circulating components in female rat plasma (4 h) following oral administration of [^{14}C]-UK-349,862

Drug-related component	Plasma radioactivity (%)	
	Scintiplates	Scintillation vials
Peak 1	16	15
Peak 2	8	7
Peak 3	33	33
Peak 4	28	29
Peak 5: UK-349,862	3	4
Peak 6	5	6

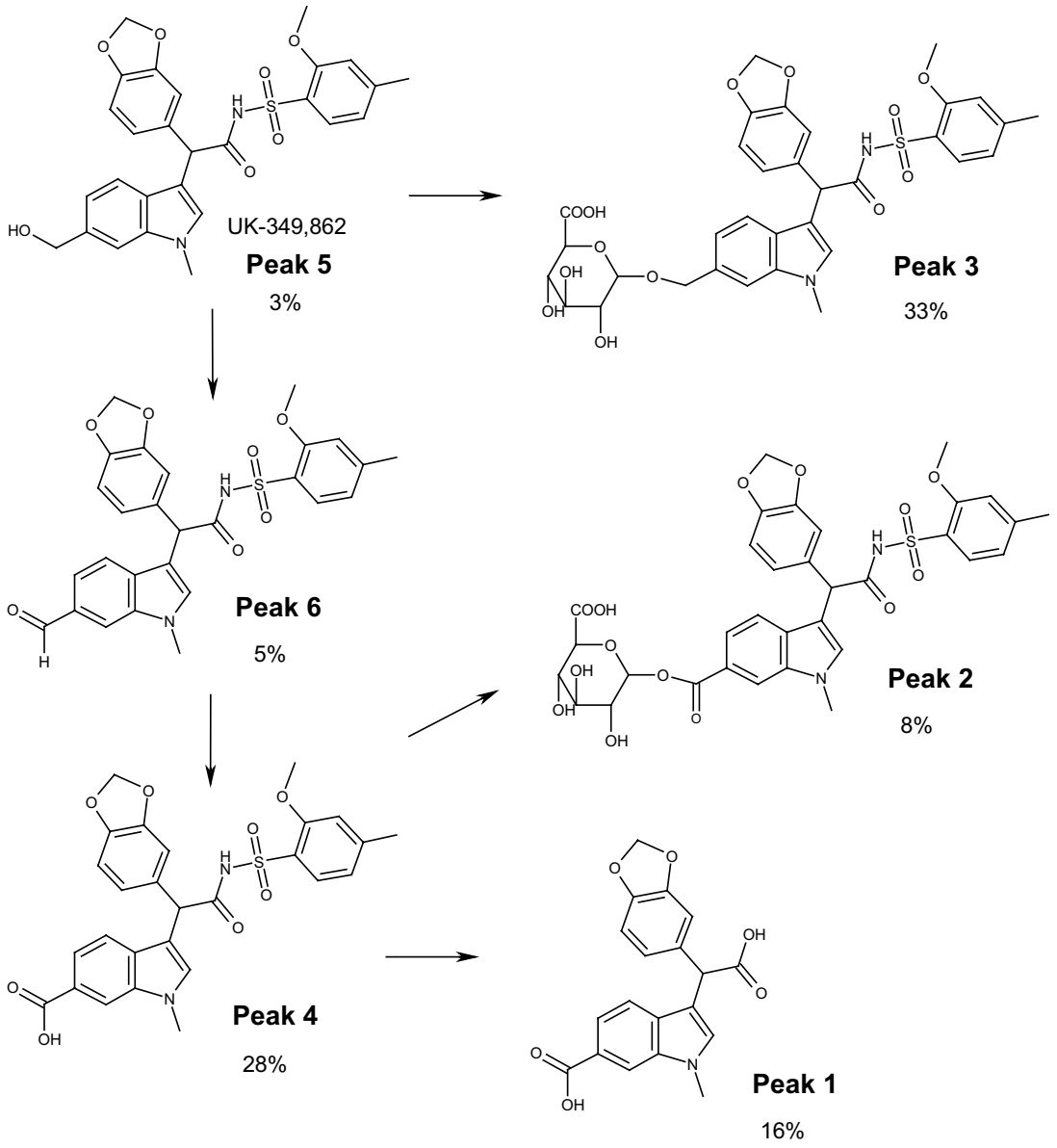


Fig. 6. Proposed metabolism scheme for female rat plasma (4 h) following oral administration of [^{14}C]-UK-349,862, showing % plasma radioactivity data.

4. Conclusions

The data presented in this study show that fraction collection into 96-well Scintiplates during HPLC profiling of radiolabelled study samples followed by off-line counting provides a significant sensitivity improvement compared to on-line radiochemical detection, whilst dramatically improving throughput compared to fraction collection into scintillation vials. Furthermore, the approach is non-destructive, such that the isolated components are available for further analysis and therefore metabolite identification and radiochemical quantitation can be achieved from a single analysis. Although the counting efficiency did

not match that achievable using liquid scintillation counting (79% compared with 90–95%), the sensitivity of the Scintiplate approach for [^{14}C] analysis was nevertheless high, such that only samples containing extremely low-levels of radioactivity will result in radiochemical profiles below the limit of detection. For excreta samples, quenching of the radiochemical signal by endogenous matrix components will in most cases make the Scintiplate approach inappropriate using either [^{14}C] or [^{3}H]. For plasma samples, however, where on-line radiochemical detection is often not possible due to low levels of drug-related material, the approach was shown to be valid for the relative quantitation of [^{14}C]-labelled material for all species under investigation. To illustrate the use of the approach for circulating metabolite profiling, a rat plasma sample following oral administration [^{14}C]-UK-349,862 was profiled by HPLC, collecting fractions into 96-well Scintiplates, which were dried and counted to yield a valid radiochemical profile of good signal-to-noise and chromatographic resolution. The drug-related components were subsequently eluted from the plates and identified by mass spectrometry. In this way, a metabolic scheme for the circulating metabolites of UK-349,862 in rat was achieved, involving relative quantitation and specific characterisation data for metabolites accounting for 93% of the total plasma radioactivity.

Historically, metabolism studies have focused on excreted components, mainly due to the low levels of drug-related material typically present in the circulation. However, it is clear that the circulating metabolites have far greater toxicological and pharmacological significance than drug-related components present in excreta. Although improvements in instrumentation and methodologies in recent years have enabled a more detailed analysis of plasma components, characterisation of circulating metabolites remains problematic in many cases. The data presented here show clearly that the use of 96-well Scintiplates is a valid, efficient and effective method for the semi-quantitative analysis and subsequent identification of metabolites present in the systemic circulation. Therefore, the approach should enhance our ability to characterise circulating metabolites and thereby improve our understanding of the safety and efficacy profile of novel development compounds.

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