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A liquid chromatography method for quantifying caffeine dissolution from pharmaceutical formulations into colloidal, fat-rich media

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ABSTRACT

A simple and rapid high-performance liquid-chromatography method is presented that permits quantification of caffeine in colloidal fat emulsions proposed as new 'biorelevant' dissolution media (Intralipid™ and various milks). Using a mobile phase of 0.1 M sodium acetate (pH 4.0) and acetonitrile (89.5:10.5, v/v) at 1 ml min^{-1}, the drug and internal standard (7-β-hydroxyethyltheophylline) were eluted within 8 min. Caffeine extraction was undertaken by protein precipitation in ice-cold 12% (w/v) trichloroacetic acid and centrifugation at 10,000 rpm for 15 min. This simple extraction method generated caffeine recovery values (corrected for % fat content) of 75.4 ± 1.4–100.6 ± 5.5%. The limit of detection was within the range 0.25–0.4 $\mu g\,ml^{-1}$ and linearity was demonstrated in each medium up to 125 $\mu g\,ml^{-1}$. Precision was <11.5% RSD and intra- and inter-day accuracy was 93.4–109.3%. The validated method was applied to *in vitro* USP dissolution tests in milk which compared the kinetics of caffeine release from (i) extended release matrices containing hydroxypropyl methylcellulose (HPMC) and (ii) an immediate release commercial analgesic tablet. Good reproducibility was obtained in both extended and immediate release dissolution tests. The method provides high-throughput quantification of this common drug in fat emulsions used as biorelevant dissolution media.

1. Introduction

Dissolution tests are used worldwide to measure the kinetics of drug release from tablets and capsules. These *in vitro* tests are useful during formulation development and are also used routinely to assure compendial compliance in the quality control of manufactured batches [1]. The potential to use dissolution tests to predict *in vivo* behaviour is an area of intense interest, as a predictive *in vitro–in vivo* correlation (IVIVC) may allow pharmaceutical companies to use dissolution testing as a surrogate for more costly human bioavailability studies [2–3].

Dissolution tests are usually undertaken in water or simple buffers, but more recently, new 'biorelevant' media have been proposed. These are designed to provide a dissolution environment which is more realistic of the fasted or fed gastro-intestinal tract [4]. Milk and other fat emulsions are popular [5–7]. A bovine milk-based medium has been proposed as best representing the contents of the fed stomach [4] following studies which demonstrated how high-fat milk (3.5% fat) displayed similar physiochemical characteristics to that of a semi-digested, FDA standardised meal [8–9]. These media are now being widely used to obtain IVIVCs for oral dosage forms that contain poorly soluble drugs [10–11].

There is a strong emerging drive to use 'biorelevant' media to predict the drug release behaviour of extended release (ER) dosage forms [12–13]. Regulatory bodies require new ER dosage forms to undergo clinical trials to demonstrate that they show similar drug release behaviour in both the fed and fasted state [14]. However, if significant differences are found, there is presently no recourse except further *in vivo* studies of a reformulated product. This is costly. Surprisingly, the interaction of dosage forms with the contents of a fed stomach is still poorly understood. Some recent evidence has suggested that the performance of some tablet technologies used in extended release dosage forms may be sensitive to certain dietary components [15–16] and the development of *in vitro* dissolution test that could predict an *in vivo* sensitivity to foods, would be a significant advance. Concurrent with developing these new dissolution tests, is a requirement to develop suitable analytical methods for the quantification of drugs in these complex emulsion-based media. These methods need to be economical,

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simple and high-throughput as high sample numbers can be generated, as ER dosage forms are dissolution tested over long time periods and in replicate.

Caffeine is an extremely common drug in commercial products, and it occurs in a wide range of cold remedies, analgesics and other types of medicines. It also possesses particularly useful attributes as a model drug with which to study the behaviour of ER dosage forms in the presence of foods: it is clinically safe, it has good pH-independent aqueous solubility in the physiological pH range (~50 mg ml^{-1} at 37 °C), photometric detection is easy, and its low partition coefficient (log P = −0.07) [17] means it will not be extensively leached from tablets by lipid components of the meal. It is therefore a useful biomarker for *in vivo–in vitro* studies in the fed state. In 'biorelevant' *in vitro* dissolution tests, it is also useful as these properties allow the drug release kinetics of an ER tablet to be studied without the competing effects of drug partitioning into the fatty phase of the emulsion dissolution medium.

In the literature, there are several older LC methods for the detection and quantification of caffeine in complex media. These include urine [18], plasma [19], green tea, other beverages [20] and certain foods [21]. More recent methods report improved sensitivity, or more rapid detection of caffeine [22–24] but there are surprisingly few published LC methods for the analysis of caffeine in fat emulsions such as milk. Most are clinical studies which focus on the separation of different xanthines, nicotine and their metabolites in breast milk [25–27], methods which are unnecessarily complex and time-consuming for routine dissolution testing. There are very few reports of quantification methods for 'biorelevant' dissolution testing, but Galia et al. [7] have measured the release of different solubility drugs from immediate release dose forms into a milk-based dissolution medium, with the aim of mimicking their *in vivo* solubility. More recently, Jantratid et al. [10] studied the release of a poorly soluble drug from lipid-filled gelatin capsules, into diluted high-fat milk, and showed that this medium could generate a predictive IVIVC. Unfortunately, these studies focused on lipid-soluble drugs, in immediate release rather than extended release dosage forms, and the reports provide little information on the accuracy and precision of the LC method used. Hence, there is a need for a quantitative, validated analytical method to study extended release and potential delayed release of a model drug such as caffeine in biorelevant, complex lipid-rich dissolution medium.

In this study, we describe a liquid chromatography method for the quantification of caffeine in fat emulsions including various milks (0.1–3.5% fat) and a high-fat emulsion, Intralipid^{TM} (30% fat). Intralipid^{TM} is a soya-bean oil/egg lecithin parenteral feeding emulsion that has been explored as a dissolution medium and is reported to have clear potential for modelling high-fat meals [5–6]. It is widely available, has highly reproducible physical characteristics and exhibits greater stability than natural high-fat foods (for example dairy cream) during the extended times required for dissolution tests of ER products [28]. An HPLC method was required to reproducibly recover caffeine from this complex fat-rich emulsion and from various milks and to investigate caffeine release during biorelevent dissolution tests of a hydroxypropyl methylcellulose (HPMC) matrix. HPMC matrices represent an ER technology of worldwide importance, and which occasionally exhibit variable behaviour in fed individuals [29–30]. Imaging studies have shown this dosage form can suffer surface fat deposition in fat emulsions, which can change the drug release mechanism and *in vitro* drug release kinetics [31]. Dissolution of commercial immediate release analgesic products, some of which show delayed drug absorption when taken with food [32–34] has not been studied in new 'biorelevant' dissolution media and hence we wished to apply new methodology to investigate an example of this type of formulation, Panadol Extra® tablets (500 mg paracetamol, 65 mg caffeine).

2. Experimental

2.1. Chemicals and materials

Low (0.1% (w/v) fat), medium (1.7%) and full-fat milk (3.5%) were freshly purchased from a local supermarket and stored at 2–8 °C. All milk-based media were used within 2 days of purchase. The parenteral emulsion, Intralipid^{TM}, which contained 30% lipid (as soya-bean oil), was a product of Fresenius Kabi (Runcorn, UK), and was kindly donated by the Sterile Productions Unit, Queens Medical Centre (Nottingham, UK). Caffeine anhydrous, 7-β-hydroxyethyltheophylline (the internal standard) and magnesium stearate were purchased from Sigma-Aldrich (Dorset, UK). HPLC-grade acetonitrile, sodium acetate and trichloroacetic acid were purchased from Fisher Scientific (Leicestershire, UK). Deionised water was used for all work and was produced by a Milli-Q Reagent Water System (USF Elga, Buckinghamshire, UK) and had a minimum resistivity of 18.2 ΩM cm. Hydroxypropyl methylcellulose (Methocel^{TM} K4M-CR Premium grade, batch no. UH22012N11) was kindly donated by Colorcon Ltd (Dartford, UK). Lactose monohydrate (fast flo) was obtained from Foremost Farms (Baraboo, USA) and microcrystalline cellulose (Avicel PH102) was obtained from the FMC Corporation (Philadelphia, USA). Panadol Extra® tablets were a product of GlaxoSmithKline (Brentford, UK) and contained 65 mg caffeine per tablet.

2.2. HPLC instrumentation

The HPLC instrumentation used in this study was a HP-1090 liquid chromatograph (Agilent Technologies, Stockport, UK) equipped with a diode array detector operating at 273 nm. The analytical column used was a Cronus C_{18} (150 mm × 4.6 mm, 5 μm particle size) and was purchased from SM-Labhut Ltd (Gloucestershire, UK). Data was collected and processed using HPLC Chemstation software (A.04.01, Agilent Technologies, Stockport, UK).

2.3. Chromatographic conditions

The mobile phase consisted of aqueous 0.1 M sodium acetate (pH 4.0) and acetonitrile in an 89.5:10.5 mixture (%, v/v). Chromatographic conditions were optimised by adjustment of organic percentage, buffer pH and buffer concentration to achieve the shortest retention time whilst providing good resolution between caffeine and internal standard peaks. The aqueous and organic phases of the mobile phase were filtered through 0.45 μm cellulose acetate and nylon filters (Whatman, Kent, UK) respectively and were helium sparged prior to use. The mobile phase flow rate was constant at 1 ml min^{-1} and all analyses were conducted at 40 °C. A 5 μl injection volume was selected for study to limit the exposure of the column to any protein or fat residue.

2.4. Sample preparation

Standard solutions (1 mg ml^{-1}) of caffeine and the internal standard were prepared by dissolving 100 mg of the active ingredient in 100 ml of the mobile phase. Solutions were stored at 2–8 °C prior to use. Dissolution test samples, calibration standards and quality control samples (all 0.4 ml) were spiked with 10 μl of the internal standard stock solution, diluted 1:2 with ice-cold 12% (w/v) trichloroacetic acid to precipitate the proteins in the milk samples and to phase-separate the parenteral emulsion. Mixtures were briefly vortexed, centrifuged at 10,000 rpm for 15 min and 0.2 ml of the clear supernatant was extracted for HPLC analysis.

Table 1
The composition of extended release matrix formulations A and B.

Ingredient (%, w/w)	Formulation A	Formulation B
Caffeine anhydrous	10.0	10.0
HPMC (Methocel™ K4M)	20.0	30.0
Lactose	49.3	39.3
Microcrystalline cellulose	19.7	19.7
Magnesium stearate	1.0	1.0

2.5. System calibration and validation

Standard solutions of caffeine (1–125 μg ml^{-1}) were prepared in different milks and Intralipid™ using the caffeine standard stock solutions. These were spiked with internal standard 10 μl and the caffeine:internal standard peak area ratio was determined at 273 nm with respect to drug concentration. The standard curves obtained were fitted to straight lines by linear regression analysis using GraphPad Instat v3.06 (GraphPad Software Inc., San Diego, CA, USA). The absolute recovery values of caffeine from each medium was determined by comparing the peak areas observed from extracted samples to respective peak areas observed from samples of corresponding strength made up in the mobile phase. This was undertaken at three different caffeine concentrations (7.5, 25 and 125 μg ml^{-1}) and performed in triplicate. Samples at three different concentrations (6.25, 25 and 125 μg ml^{-1}) were prepared in each medium when assessing the intra-day (six assays on a single day) and inter-day (six assays on three separate days) variation of the method. The limit of detection (LOD) and limit of quantification (LOQ) for caffeine were determined in each medium and were defined as the lowest concentration which could generate signal-to-noise ratios of 3:1 and 9:1 respectively.

2.6. Determination of the volume of aqueous phase in Intralipid™ 30%

The volume of aqueous component in Intralipid™ 30% was determined by thermogravimetric analysis using a MB45 Moisture Analyser (Ohaus Corporation, Florham Park, US). 2 ml of Intralipid™ was transferred to a tared aluminium pan and placed within the moisture balance. The temperature rapidly increased to 175 °C, and then slowly reduced to a target temperature of 125 °C. Change in sample mass during heating was indicative of a loss of sample moisture, and was continuously monitored by the balance. The test automatically terminated following no change in mass over 30 s. The loss of sample mass was representative of the quantity of aqueous phase within the Intralipid™. This method showed excellent reproducibility (RSD < 0.1%). The aqueous phase in Intralipid™ 30% was determined by this method to be 67.1% (w/w) (N = 5) a figure comparable to the theoretical value of 67.4% (v/v) which can be calculated using the density of soya-bean oil (0.92 g ml^{-1}).

2.7. Manufacture of extended release HPMC matrices

The compositions of the extended release HPMC matrix tablets used in these studies are shown in Table 1. Formulation A contained the lowest HPMC content, and was designed to show a faster drug release profile compared with Formulation B. Matrices were manufactured by compression into flat-faced, 8 mm diameter tablets of 250 ± 5 mg mass on an instrumented F3 tablet press (Manesty, Liverpool, UK) at 240 MPa. Preliminary unpublished studies had shown that tablet drug content uniformity was within 95–105% of the label claim.

Table 2
Recovery efficacy for caffeine in different media.

Medium	Spiked caffeine concentration (μg ml^{-1})	Recovery[a] (%)	Corrected recovery (%)[b]
Low-fat milk (0.1% fat)	7.5	100.6 ± 5.5	
	25.0	99.8 ± 1.3	
	125.0	97.2 ± 0.8	
Medium-fat milk (1.7% fat)	7.5	96.5 ± 2.6	
	25.0	97.8 ± 2.1	
	125.0	97.2 ± 1.1	
Full-fat milk (3.6% fat)	7.5	95.1 ± 4.8	
	25.0	98.1 ± 0.2	
	125.0	97.4 ± 1.7	
Intralipid™ 30% (30% fat)	7.5	125.5 ± 2.1	84.2
	25.0	112.3 ± 1.4	75.4
	125.0	114.2 ± 0.4	76.6

[a] Recovery values determined by comparing caffeine peak area in the mobile phase samples and peak area in the emulsions.
[b] Recovery values are corrected for the amount of aqueous phase in Intralipid™.
Recovery values are expressed as mean (N = 3) ± 1 s.d.

2.8. Dissolution testing

The drug release characteristics of HPMC matrices and Panadol Extra® tablets in 900 ml dissolution media (37 ± 0.5 °C) were studied using a USP dissolution testing apparatus 1 (Dissolutest, Prolabo, France) at a basket rotation speed of 100 rpm. 3 ml aliquots of dissolution medium were removed at intervals from each dissolution vessel using a plastic syringe attached to a filtered stainless-steel cannula, and replaced with fresh medium. Care was taken to ensure that samples were removed from the same area of the dissolution vessel each time, namely the midpoint between the top of the rotating basket and the surface of the dissolution medium. 0.4 ml aliquots were removed from the dissolution samples and caffeine concentration was determined by the HPLC method described above.

Drug release profiles of the two different HPMC formulations were compared using the similarity factor f_2 calculated using Eq. (1):

$$f_2 = 50 \log \left\{ \left[1 + \frac{1}{n} \sum (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\} \quad (1)$$

where n is the number of time points analysed, R_t the mean amount of drug released from the reference formulation (A) and T_t is the mean amount of drug release from the test formulation (B). Using this equation, a f_2 value between 50 and 100 is required for release profiles to be sufficiently similar for bioequivalence [35].

3. Results and discussion

3.1. Chromatography

Fig. 1 shows representative chromatograms of the blank media at different fat concentrations, and when spiked with caffeine and the internal standard (7-β-hydroxyethyltheophylline). Blank chromatograms showed no significant interference after ~4 min of each run. Retention times for the caffeine and the internal standard were 4.8 and 7.1 min respectively. There was no evidence of any additional peaks (up to 15 min), suggesting that caffeine and the internal standard did not decompose in these media. As a result, an 8 min total run time was used.

3.2. Sample extraction and recovery

Table 2 shows the recovery of caffeine from different colloidal fat media using the standard extraction procedure. The recovery

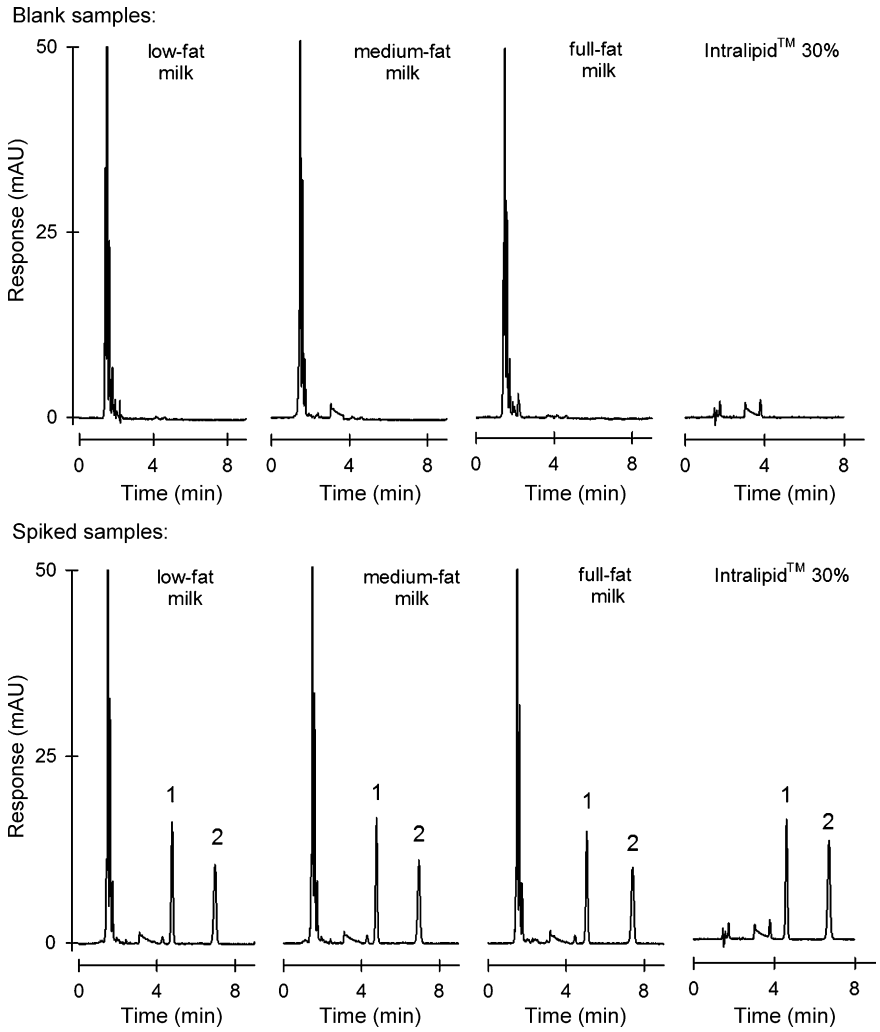

Fig. 1. Chromatograms of blank (upper) or spiked (lower) milk and Intralipid™ 30% samples extracted with 12% trichloroacetic acid. Samples were spiked with internal standard (peak 1) and caffeine (peak 2). The mobile phase was 0.1 M sodium acetate (adjusted to pH 4) in a v/v ratio of 89.5:10.5 with acetonitrile. Flow rate 1 ml min^{-1}. Injection volume 5 μl. Analysis conducted at 40 °C.

of caffeine from the milk-based media was both efficient (>95%) and highly reproducible (RSD < 2%) but showed greater variation at the lowest caffeine concentration studied (7.5 μg ml^{-1}: RSD < 6%). In Intralipid™ 30% the calculated caffeine recovery was consistently over 100%, and this was attributed to the decreased aqueous phase volume in these fat-rich samples. The high aqueous solubility and low log *P* means that the majority of the caffeine would be located in the aqueous phase. The aqueous phase in Intralipid™ was determined to be 67.1% (w/w) (section 2.6) and recovery values for caffeine in Intralipid™ samples were corrected using this value. Corrected recoveries are shown in Table 2 and ranged from 75.4% and 84.2%. These values are consistent with the partition coefficient of caffeine (log *P* = −0.07) which predicts that ~15% will leach into the lipid phase. Corrected recoveries for the internal standard (also water-soluble) ranged from 74.2 ± 0.3%–94.8 ± 2.5%, and were comparable to those observed for caffeine which confirmed the suitability of 7-*β*-hydroxyethyltheophylline as an internal standard in this method. Acetonitrile has also been used in the literature to extract caffeine and other drugs from milk [11,27]. Surprisingly however, when added to the samples in our experiments, acetonitrile appeared to cause peak splitting of the internal standard (not shown), perhaps due to the difference in solvent polarity between acetonitrile and the predominantly aqueous mobile phase. The effect of acetonitrile was also observed for the structurally related compounds, theophylline and theobromine (which showed retention times comparable to that of the internal standard) and this confirmed our preference for using the acid precipitation method. The extraction efficiency in this study of caffeine from milks was comparable to or better than those in the recent literature [25–26]. Caffeine extraction from Intralipid™ has, to the best of our knowledge, not been previously reported in the literature, and we unaware of other studies which have validated a method for the extraction of a water-soluble drug from complex emulsions containing up to 30% fat. Caffeine LOD values in different media were found to be between 0.25–0.4 μg ml^{-1} whilst the LOQ ranged from 0.6–0.8 μg ml^{-1}. These values were obtained using a 5 μl injection volume, and the LOQ was deemed to be satisfactory for this work as the minimum caffeine concentrations observed during *in vitro* dissolution testing were likely to be above 1 μg ml^{-1}. However, it was possible to increase the sensitivity of the method to caffeine by increasing the injection volume, and a 9:1 signal-to-noise ratio was achieved at 0.5 μg ml^{-1} using a 7.5 μl injection volume. The measured values of sensitivity were comparable with those reported for caffeine in other complex media [36–38].

3.3. System calibration and validation

Table 3 shows correlation coefficient values (r^2) of the regression line fitted to the standard curves obtained in each medium, and shows the intra- and inter-day variability. In each medium, the 10-

Table 3
Linearity (r^2), intra-day ($N = 6$) and inter-day ($N = 3$) accuracy and precision for the caffeine assay using different media.

Medium	Calibration equation (r^2)	Spiked caffeine concentration ($\mu g\,ml^{-1}$)	Accuracy (%)		Precision (RSD)	
			Intra-day	Inter-day	Intra-day	Inter-day
Low-fat milk (0.1% fat)	$y = 0.0409x + 0.0882$ (0.9989)	6.25	93.4	99.1	5.11	10.73
		25.0	98.7	98.7	5.23	4.26
		125.0	101.4	100.3	4.75	7.48
Medium-fat milk (1.7% fat)	$y = 0.0438x + 0.0504$ (0.9998)	6.25	98.1	106.4	1.96	11.38
		25.0	102.5	101.9	1.66	1.21
		125.0	100.0	101.8	3.77	8.21
Full-fat milk (3.6% fat)	$y = 0.0409x - 0.0229$ (0.9998)	6.25	100.0	104.0	2.83	6.58
		25.0	100.8	101.1	2.26	1.98
		125.0	100.3	105.8	0.46	4.37
Intralipid™ 30% (30% fat)	$y = 0.0401x - 0.0339$ (0.9998)	6.25	103.6	107.4	3.48	4.88
		25.0	100.8	109.3	2.26	4.77
		125.0	103.9	106.7	3.59	4.17

point calibration curves showed good linearity ($r^2 \geq 0.999$) within a drug concentration range of 1–125 $\mu g\,ml^{-1}$. This concentration range is suitable for dissolution testing. The gradients were comparable in different media and there was good intra-day and inter-day accuracy (93.4–102.5% and 98.7–109.3%, respectively). Intra-day and inter-day precision at 25 $\mu g\,ml^{-1}$ and 125 $\mu g\,ml^{-1}$ was good (RSD < 8.21%) but showed greater variability at 6.25 $\mu g\,ml^{-1}$ (RSD < 11.38%). The accuracy and precision of the HPLC assay showed no dependence on the fat concentration in the medium. This validation provided confidence that the method was suitable for quantifying drug release in the subsequent dissolution tests.

3.4. Application of the method to studies of caffeine release in colloidal fat media

Dissolution tests were undertaken to compare the extended release properties of two HPMC matrix formulations (Table 1) in full-fat milk. An example chromatogram obtained from a dissolution test sample is shown in Fig. 2(a). The results of the dissolution tests are shown in Fig. 3, plotted as the cumulative increase in caffeine concentration ($\mu g\,ml^{-1}$) with respect to time and the horizontal dotted line shows the theoretical maximum caffeine concentration that would be reached if 100% release of drug was achieved. Both formulations exhibited extended release properties over approximately 12 h, by which time the caffeine concentration in the medium had reached 95% to 105% of the theoretical maximum. Standard deviations were at worst ±5.3% and more normally were ±4% for single points on the mean curve (Fig. 3). These values are within the range normally expected in dissolution tests of these products where drug is quantified by UV spectrometry. As might be expected, Formulation A containing 20% polymer, exhibited faster drug release kinetics than Formulation B containing 30% polymer. A simple comparison between these curves can be made using the time taken to achieve 50% release (T_{50}%). The mean T_{50}% value for Formulation A was 1.97 ± 0.17 h and for Formulation B was 2.95 ± 0.08 h. Once again, there is sufficient reproducibility here to allow discrimination of the two curves. A more sophisticated statistical comparison using a standard method used for dissolution testing (described in section 2.8) gave an f_2 value of 49.8. This value is just below the threshold value of 50 that would classify these formulations as sufficiently similar to be bioequivalent, and avoid the need for a clinical study [35].

Dissolution tests to compare the rate of caffeine release from a commercial immediate release tablet (Panadol Extra®) were under-

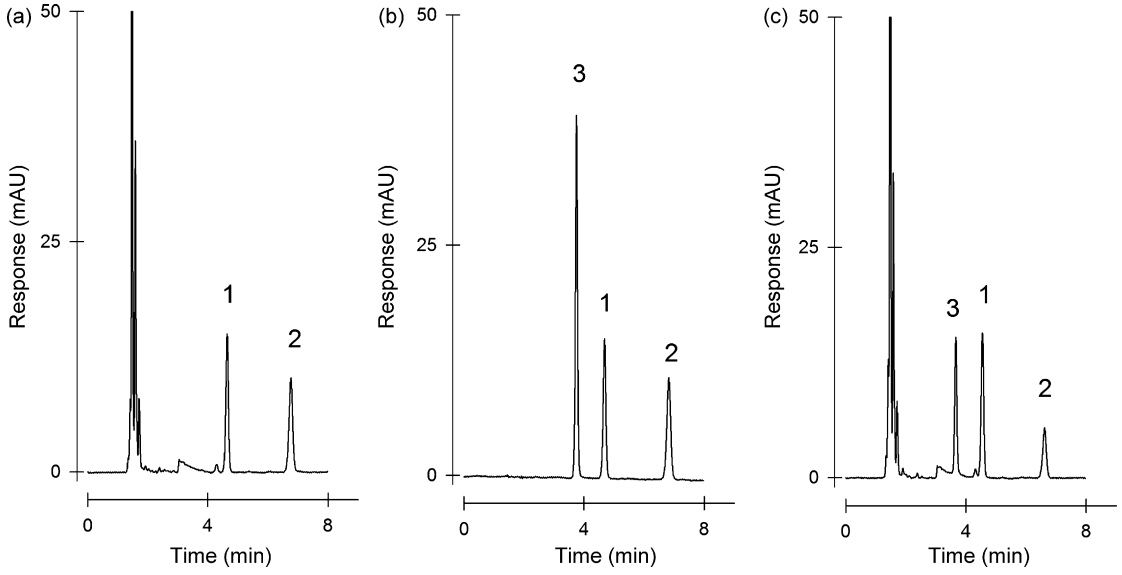

Fig. 2. Chromatograms of samples obtained during the *in vitro* dissolution testing of caffeine-containing solid dosage forms. Samples correspond to (a) Formulation A after 6 h dissolution in full-fat milk, or Panadol Extra® tablets after 5 min dissolution in (b) water and in (c) full-fat milk. Peak 1 = internal standard, peak 2 = caffeine and peak 3 = paracetamol (not quantified).

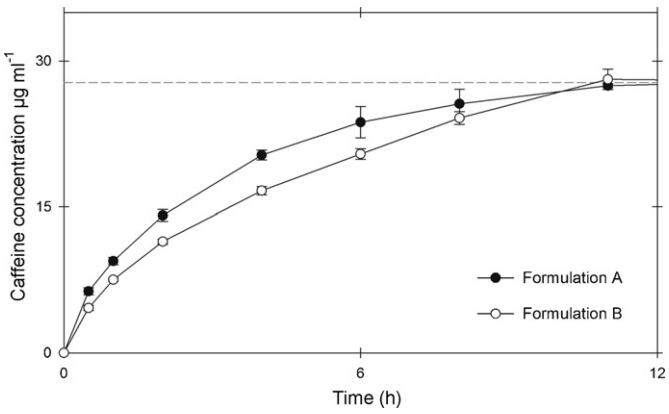

Fig. 3. Release of caffeine from extended release HPMC matrices in full-fat milk dissolution media. Formulations A and B contain 20% and 30% HPMC respectively (the full compositions are shown in Table 1). Dissolution tests were undertaken in USP apparatus 1, 100 rpm, 900 ml medium at 37 ± 0.5 °C. Mean (N = 4) ± 1 s.d. The horizontal dotted line denotes the theoretical maximum caffeine concentration at 100% drug release which was 25 mg in 900 ml.

taken in water and in full-fat milk. Example chromatograms from the dissolution test samples are shown in Fig. 2b and c, and the drug release profile is shown in Fig. 4. In water, ~80% caffeine release was evident after 20 min. This is a suitably rapid rate of tablet disintegration and drug dissolution for an immediate release product. However in milk, this tablet showed significantly retarded drug release, with only ~40% of the caffeine being released after 20 min. HPLC chromatograms in Fig. 2b and c provided further evidence for this slower drug release; there was a visible reduction in peak height of both caffeine (peak 2) and paracetamol (peak 3) in milk samples. After 60 min, there was negligible change in the caffeine concentration in both media which suggested that complete tablet disintegration had occurred (Fig. 4). Maximum caffeine concentrations observed did not reach the theoretical maximum (72.2 µg ml^{-1}), but were within 10% of tablet label claim reaching 65.8 µg ml^{-1} after 45 min in water, and 70.2 µg ml^{-1} after 90 min in milk.

The work presented above gives confidence that the HPLC method developed in this paper can provide good discrimination between the behaviour of different products in dissolution tests conducted in water and in full-fat milk. The results of these investigations were consistent with previous studies, which show the same relationship between drug release and polymer content in HPMC matrices [39–40] and reduced rates of drug release from immediate release analgesic tablets in the presence of milk [41]. Reproducibility of the dissolution tests was considered satisfactory, as the standard deviation bars in Figs. 3 and 4, were similar to those obtained by simple UV spectrometry during routine dissolution testing in aqueous media.

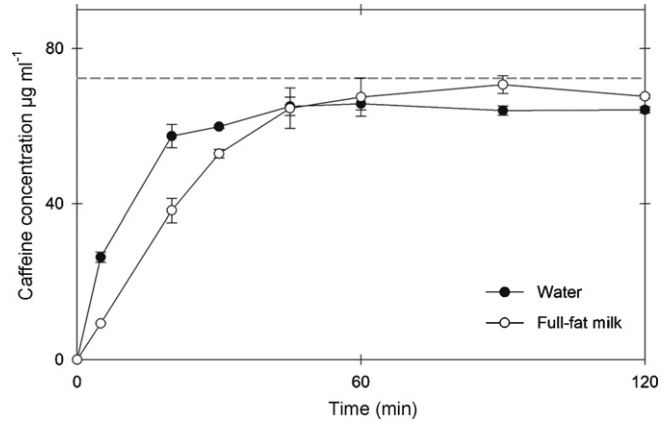

Fig. 4. Release of caffeine from immediate release Panadol Extra® tablets in water or full-fat milk. Dissolution tests were undertaken in USP apparatus 1, 100 rpm, 900 ml medium at 37 ± 0.5 °C. Mean (N = 3) ± 1 s.d. The horizontal dotted line denotes the theoretical maximum caffeine concentration at 100% drug release which was 65 mg in 900 ml.

4. Conclusions

There is currently a strong drive to develop *in vitro* dissolution tests that are more 'biorelevant'. Milks and high-fat emulsions are presently the dissolution media of choice and are considered to be representative of the fed state. In this study, an LC method was developed for the detection and quantification of caffeine in these complex media. The method yielded caffeine recovery values from milk which were equal or superior to those recently described [25–26], and which was applicable to a high-fat parenteral emulsion (Intralipid™ 30%). The method was applied successfully in dissolution studies that assessed the drug release performance of extended release HPMC matrices and a typical immediate release commercial analgesic product, both of which have been reported to be sensitive to fed state interactions. The method appears to be suitably simple, cost-effective, efficient and high-throughput for the routine dissolution testing of caffeine-containing dosage forms in these complex colloidal emulsions. It is hoped that the method presented here and those developed elsewhere in response to this work, might encourage the more routine *in vitro* testing of solid dosage forms in complex media representative of the fed state. Such tests can only improve our understanding of their *in vivo* behaviour, and might assist in the development of more robust delivery systems. The method may have wider applicability to other commercial products containing caffeine, and have possible application in the quantification of caffeine in other complex, lipid-based emulsion systems such as nutrient-enriched drinks, milkshakes and semi-digested foods. Caffeine is a highly useful model drug and a safe biomarker, and this method might also be useful in the design and *in vitro* assessment of existing and novel extended release technologies in biorelevant media.

References

[1] US Food and Drug Administration, Guidance for Industry: Extended Release Oral Dosage Forms, Development Evaluation and Application of In Vitro/In Vivo Correlations, Centre for Drug Evaluation and Research (CDER), Rockville, 1997.
[2] US Food and Drug Administration, Guidance for Industry: SUPAC-IR/MR: Immediate Release and Modified Release Solid Oral Dosage Forms, Manufacturing Equipment Addendum, Centre for Drug Evaluation and Research (CDER), Rockville, 1999.
[3] US Food and Drug Administration, Guidance for Industry: Bioavailability and Bioequivalence Studies for Orally Administered Drug Products, G. Considerations, Centre for Drug Evaluation and Research (CDER), Rockville, 2002.
[4] E. Jantratid, N. Janssen, C. Reppas, J.B. Dressman, Pharmaceutical Research 25 (2008) 1663.
[5] B. Abrahamsson, K. Roos, J. Sjogren, Drug Development and Industrial Pharmacy 25 (1999) 765.
[6] L.J. Ashby, A.E. Beezer, G. Buckton, International Journal of Pharmaceutics 51 (1989) 245.
[7] E. Galia, E. Nicolaides, D. Horter, R. Lobenberg, C. Reppas, J.B. Dressman, Pharmaceutical Research 15 (1998) 698.
[8] S. Klein, J. Butler, J.M. Hempenstall, C. Reppas, J.B. Dressman, Journal of Pharmacy and Pharmacology 56 (2004) 605.
[9] L. Kalantzi, K. Goumas, V. Kalioras, B. Abrahamsson, J.B. Dressman, C. Reppas, Pharmaceutical Research 23 (2006) 165.
[10] E. Jantratid, N. Janssen, H. Chokshi, K. Tang, J.B. Dressman, European Journal of Pharmaceutics and Biopharmaceutics 69 (2008) 776.
[11] Y. Shono, E. Jantratid, N. Janssen, F. Kesisoglou, Y. Mao, M. Vertzoni, C. Reppas, J.B. Dressman, European Journal of Pharmaceutics and Biopharmaceutics 73 (2009) 107.
[12] K.A. Lentz, AAPS Journal 10 (2008) 282.
[13] S. Klein, M.W. Rudolph, B. Skalsky, H.U. Petereit, J.B. Dressman, Journal of Controlled Release 130 (2008) 216.
[14] US Food and Drug Administration, Guidance for Industry: Food-Effect Bioavailability and Fed Bioequivalence Studies, Centre for Drug Evaluation and Research (CDER), Rockville, 2002.

[15] H.D. Williams, R. Ward, I.J. Hardy, C.D. Melia, Journal of Controlled Release 138 (2009) 251.
[16] H.D. Williams, R. Ward, I.J. Hardy, C.D. Melia, Journal of Pharmacy and Pharmacology 60 (2008) 115.
[17] A.C. Moffat, M.D. Ossepton, B. Widdop, Clarke's Analysis of Drugs and Poisons, 3rd ed., Pharmaceutical Press, London, 2004.
[18] A.W. Abu-Qare, M.B. Abou-Donia, Journal of Pharmaceutical and Biomedical Analysis 26 (2001) 939.
[19] C. Pistos, J.T. Stewart, Journal of Pharmaceutical and Biomedical Analysis 36 (2004) 737.
[20] J.L. Blauch, S.M. Tarka, Journal of Food Science 48 (1983) 745.
[21] H. Terada, Y. Sakabe, Journal of Chromatography 291 (1984) 453.
[22] N.N. Atia, P. York, B.J. Clark, Journal of Separation Science 32 (2009) 931.
[23] J.P. Naik, S. Nagalakshmi, Journal of Agricultural and Food Chemistry 45 (1997) 3973.
[24] H. Horie, A. Nesumi, T. Ujihara, K. Kohata, Journal of Chromatography A 942 (2002) 271.
[25] M. Pellegrini, E. Marchei, S. Rossi, F. Vagnarelli, A. Durgbansh, S. Garcia-Algar, O. Vall, S. Pichini, Rapid Communications in Mass Spectrometry 21 (2007) 2693.
[26] A. Aresta, F. Palmisano, C.G. Zambonin, Food Chemistry 93 (2005) 177.
[27] C.M. Berlin, H.M. Denson, C.H. Daniel, R.M. Ward, Pediatrics 73 (1984) 59.
[28] A.G. Floyd, Pharmaceutical Science & Technology Today 2 (1999) 134.
[29] B. Abrahamsson, M. Alpsten, B. Bake, U.E. Jonsson, M. Eriksson-Lepkowska, A. Larsson, Journal of Controlled Release 52 (1998) 301.
[30] J. Davis, J. Burton, A.L. Connor, R. Macrae, I.R. Wilding, Journal of Pharmaceutical Sciences 98 (2009) 1568.
[31] H.D. Williams, K.P. Nott, R. Ward, I.J. Hardy, C.D. Melia, Journal of Pharmacy and Pharmacology 61 (2009) 2.
[32] M. Divoll, D.J. Greenblatt, B. Ameer, D.R. Abernethy, Journal of Clinical Pharmacology 22 (1982) 571.
[33] S. Souliman, S. Blanquet, E. Beyssac, J.M. Cardot, European Journal of Pharmaceutical Sciences 27 (2006) 72.
[34] J.M. Jaffe, J.L. Colaizzi, H. Barry, Journal of Pharmaceutical Sciences 60 (1971) 1646.
[35] J.W. Moore, H.H. Flanner, Pharmaceutical Technology 20 (1996) 64.
[36] R. Ventura, C. Jimenez, N. Closas, J. Segura, R. De la Torre, Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences 795 (2003) 167.
[37] M.D.R. Brunetto, L. Gutierrez, Y. Delgado, M. Gallignani, A. Zambrano, A. Gomez, G. Ramos, C. Romero, Food Chemistry 100 (2007) 459.
[38] M.C.R. De Camargo, M.C.F. Toledo, Journal of the Science of Food and Agriculture 79 (1999) 1861.
[39] K.C. Sung, P.R. Nixon, J.W. Skoug, R.T.C. Ju, P. Gao, E.M. Topp, M.V. Patel, International Journal of Pharmaceutics 142 (1996) 53.
[40] M.V. Velasco, J.L. Ford, P. Rowe, A.R. Rajabi-Siahboomi, Journal of Controlled Release 57 (1999) 75.
[41] S. Anwar, J.T. Fell, P.A. Dickinson, International Journal of Pharmaceutics 290 (2005) 121.