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A comparative bioavailability study of different aspirin formulations using on-line multidimensional chromatography

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Abstract

A multi-dimensional column chromatographic method employing UV spectrometric detection was optimised and successfully used in a comparative bio-availability study of aspirin obtained from different commercially available oral dosage forms. Sample clean-up was achieved by on-line solid-phase extraction. In this study, the bioavailability of aspirin was compared in plain aspirin tablets, chewed tablets, effervescent tablets and Enteric-coated aspirin tablets. Blood samples were taken at frequent intervals after single dosing in ten healthy volunteers, the plasma samples were first treated with physostigmine sulphate to minimise enzymatic hydrolysis of aspirin to salicylate. The results showed the measured T_{max}, C_{max} and AUC was significantly higher for soluble aspirin than for the other formulations and the $t_{1/2}$ was shorter. This indicates the rapid absorption of aspirin from a soluble formulation compared with that from the other formulations. These differences suggest that the soluble formulation could be the aspirin of choice to treat patients suspected to be at high risk of myocardial infarction. The method performs, in a single step, an efficient extraction and clean-up of aspirin from human plasma. The calibration graph was linear over the calibration range 0.2–12 μg ml^{-1} plasma with a limit of detection of 0.1 μg ml^{-1}. The intra- and inter-assay coefficients of variation were less than 6% and the recoveries ranged from 86 to 98%. The proposed method combines the advantages of being simple and selective in the presence of other potential interfering drugs and is suitable for routine analyses to obtain valuable information about the clinical effects of the drug and its use in prevention treatments of acute myocardial infarction. The whole procedure takes ~7 min and is in agreement with other conventional methods.

Keywords: Multi-dimensional chromatography; Aspirin; Bioavailability studies; Pharmaceutical formulations

1. Introduction

Aspirin (Fig. 1) is the salicylate ester of acetic acid [I]. It is the protype of the salicylates, and is a non-steroidal anti-inflammatory agent (NSAIA). The drug is rapidly and widely dis-

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tributed into most body tissues and fluids. The volume of distribution of aspirin is approximately the same as that of salicylate and is generally 15–21 l kg^{-1}. The drug is hydrolysed in-vivo to give salicylic acid [II].The ability of aspirin to acetylate proteins (e.g. platelet proteins, hormones, DNA, haemoglobin) results in some therapeutic effects, such as inhibition of platelet aggregation, which other currently available salicylates do not exhibit. The use of aspirin as an antiplatelet agent has been discussed in several reviews [1–5]. Generally accepted indications for prophylactic aspirin therapy include its use for reducing the risk of recurring transit ischematic attacks (TIAs) and stroke or death in man who had single or multiple TIAs and, in low doses, reducing the risk of recurrent myocardial infarction and/or death in patients with myocardial infarction, at least in the acute phase.

The American College of Cardiology (ACC), American Heart Association (AHA), and many clinicians currently recommend low-dose aspirin therapy (160–325 mg day^{-1}) for prevention of early and late infarction and death following acute myocardial infarction in all patients who can tolerate the drug, regardless of whether thrombolytic therapy has been administered [6–11]. Other results from clinical trials suggest that aspirin may be useful for the prevention of certain forms of thrombolic arterial disease [12–14].

These recent medical uses of the drug require the need for assay methods in biological fluids to study its clinical effect and assist in the selection of the suitable formulation to be used in such treatment courses. Previously used methods involve the extraction of the drug into an organic layer, evaporating the organic layer to dryness under vacuum at 45°C, then reconstituting in 100 ml mobile phase and injecting an aliquot of the reconstituted volume [15–22]. Off-line liquid–liquid extraction procedures may cause loss of the analyte through adsorption of the drug onto glassware during the extraction procedures. Adsorptive and evaporative losses may also occur during the solvent extraction or following solvent removal 'blow down' step, when the analyte is allowed to form a dry residue on the inside surface of the container [23]. The procedures can also take up to 1 hr before injection of the sample. The column switching technique described in this study affords an interesting and creative alternative for sample preparation. The method involves a backflush of the second column after the targeted compound has been trapped at the inlet of the second column. The method has successfully separated the drug from the other components of plasma and its main metabolite, salicylic acid (SA), and was applied to investigate a comparison of drug release and bio-availability from four well-formulated different commercially available aspirin dosage forms with respect to peak concentration and time of appearance of the drug in the plasma after oral dose. Such a comparison can be of primary importance for correct prevention or treatment of acute myocardial infarction and other forms of thrombolic arterial diseases.

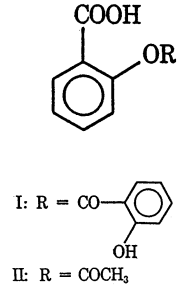

Fig. 1. Basic structure of aspirin (I) 'acetyl salicylic acid' (ASA) and salicylic acid(II) 'AS'.

2. Study plans and methodology

2.1. Materials and reagents

1-Butanol (Analar grade), HPLC grade methanol, physostigmine sulphate, di-*n*-butylamine and codeine phosphate were supplied by Sigma (Poole, Dorset, UK). Analytical grade orthophosphoric acid was supplied by BDH (BDH Ltd., Poole, Dorset, UK). Water was distilled and then further purified by passing through a Milli-Q water purification system (Millipore, Milford, MA). Plasma samples were obtained from normal human volunteers. Heparinized plastic tubes 'Li-Heparin Monovette', were supplied by Sarstedt

(Numbrecht, Germany). Caffeine, acetaminophen, phenacetin and salicylamide were supplied by Aldrich (Dorset, UK). The four different dosage forms included in the comparison were: 650 mg plain aspirin tablets (2 × 325 mg, taken whole with water, Empirin®, Burroughs, Welcome), 600 mg chewed tablets (2 × 300 mg, Dispirin®, Direct tablets), 650 mg effervescent tablets dissolved in water (2 × 325 mg, Alka-Seltzer®, Miles), 500 mg Enteric-coated tablets (Ecotrin®, Smithkline Beecham).

2.2. Instrumentation and operating conditions

The chromatographic conditions used in the analysis is based on the work of Brandon et al [19], following a slight modification in the methanol:water ratio and the flow rate to optimise the retention time and peak width for aspirin. The drug and plasma extracts were separated on a 30 cm × 3.9 mm μBondapak 10 μm C_{18} (octadecylsilane), obtained from Waters (Milford, HA). The column temperature was maintained at 47°C using a Tracor Instruments column heating Jacket (Austin, TX). The analytical column was protected by a guard column (23 cm × 3.9 mm interior diameter) packed with μBondapak C_{18} /Porasil B (Waters). The mobile phase consisted of MeOH:Water:1-Butanol:orthophosphoric acid, 350:640:10:0.13. Prior to use, the mobile phase was filtered with a 0.45-μm HA filter Millipore and degassed under reduced pressure. The prepared eluent was delivered by a Waters Model 501 HPLC pump (pump B) at a rate of 2 ml min^{-1} (2800 psi). Before use, the analytical column was primed with di-*n*-butylamine to avoid tailing of the salicylic acid (SA) peak. For this purpose, 400 μl di-*n*-butylamine was added to 200 ml eluent, and the mobile phase was recycled overnight at a flow-rate of 0.3 ml min^{-1}. Sample introduction was via a Rheodyne (Cotate, CA) Model 7125 injection valve, fitted with a 25 μl loop for direct injection. For the purposes of extraction by column switching, the injector was fitted with a 1 ml loop and a second pump (pump A) and the concentration column were connected to the analytical assembly via a Rheodyne Model 7000 six-port switching valve. The concentration column (10 cm × 1.5 mm i.d.) was dry-packed in-house with Corasil (Waters) C_{18} (37 μm) packing material. The peaks for the compound were detected using a Shimadzu SPD-6A variable-wave length UV detector. UV absorbance was measured at 254 nm, and the peak heights were recorded using Philips Model PM 8261 chart recorder, set at 0.04 aufs and chart speed was 60 cm h^{-1}. At the end of each session, the column was washed with water for 30 min, and re-equilibrated for 20 min. Such a washing cycle is desirable for the potential removal of plasma matrix factors that might accumulate in the system.

2.3. Bioavailability studies

Bioavailability studies usually are conducted in normal healthy adults under standardised conditions. The goal of such studies is to evaluate the performance of the dosage forms. The protocol should define the acceptable weight range for the subjects to be used. Ten healthy fasted male volunteers in the age range 30–60 years were selected for the study, the volunteers weighing between 60 and 90 kg. None of the volunteers was under medication that might interfere with the release or detection of aspirin. Venous blood samples were collected into prechilled heparinized plastic tubes, chilled in ice and centrifuged (10 min, 3000 × *g*, 4°C) to separate the sample. After collection, plasma was first treated with physostigmine sulfate to minimise enzymatic hydrolysis of aspirin to salicylate.

2.4. Standard solutions and calibration curves

Stock solutions equivalent to 0.02 mg ml^{-1} of aspirin were freshly prepared in deionised water. These were diluted and added to drug-free plasma standards in the concentration range 0.2–12 μg ml^{-1}. Each calibration point was run in triplicate over 3 consecutive days. All solutions were stored at 4°C and protected from light. Under these conditions, decomposition of ASA to SA was less than 2% within 1 month.

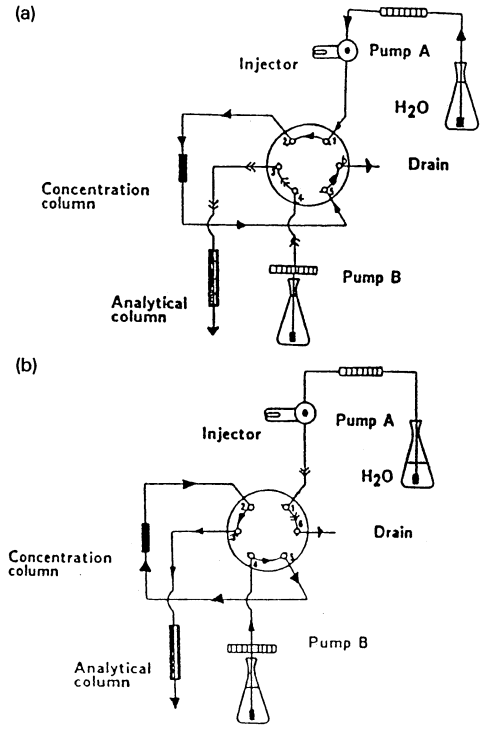

Fig. 2. Column switching assembly, used for retaining aspirin in the preconcentration column (a) and eluting the drug from the pre-concentration column (b).

2.5. Extraction and detection procedures

Acetyl salicylic acid was extracted from the biological using on-line solid phase extraction with column switching, illustrated in Fig. 2. Aliquots of the drug in plasma (1 ml) were introduced via the injector port and swept onto the concentration column by water from pump A, whereupon the gross plasma interferents were eluted to waste, while the drug was retained on the analytical column (a). After a predetermined washtime, the valve was rotated to position 2, which caused the analytical mobile phase, delivered by pump B, to flow in a back-flush mode through the concentration column hence the retained drug was swept onto the analytical column for separation (b). The analyte was detected using UV detector, set at 254 nm, 0.04 aufs.

2.6. Examination of interferences

Under the above optimised conditions, a number of compounds were screened to evaluate their interferences on the assay of aspirin. This included codeine phosphate, caffeine, acetaminophen, phenacetin, salicylamide and the hydrolytic metabolite, salicylic acid. The effect of these substances in the proposed method was studied by the addition of such drugs to the plasma samples already spiked with aspirin at concentration-levels similar to those of the drug (10 μg ml^{-1}).

3. Results and discussion

3.1. Optimisation of separation and pre-concentration conditions

Selective pre-concentration may be defined as the collection, separation, and enrichment of a single chemical component (or class of compounds). It is usually required to increase the sensitivity and enhance the selectivity of a measurement system, and to minimise loading of the sensor by extraneous material. A column switching set-up was selected to achieve these requirements.

The first-step in the set-up of the switching system involved the selection of a suitable pre-concentration packing material and a column that can retain the drug after washing any endogenous interferents. A short stainless steel pre-column (10 mm × 1.5 mm I.D) and pellicular C_{18} packing material showed the most favourable washing and retention ability when compared with other conditions and was therefore chosen for washing and pre-concentration purposes.

The next step is to find a washing system that contains an eluent which has the ability to wash any interferents, while retain the drug in the pre-concentration column until the washing is finished. Such an eluent should also be compatible with the mobile phase, as even slight incompatibility could result in a slug of solvent travelling down the analytical column partially trapping sample components and can cause band broaden-

ing leading to a poor chromatographic results. Various eluents with a different elutropic strengths were examined, and degassed deionised water was found to be adequate to provide the necessary washing of endogenous components without the loss of the analyte; this washing eluent was also compatible with the analytical mobile phase. Finally, an optimum wash time interval, that would be enough to clean-up the plasma components through the pre-concentration column without causing the elution of the drug, had to be determined in a reasonable time. The wash time (defined as the length of time between injection and switching of the valve) was varied between 1 and 5 min, and it was found that a maximum cleaning and a minimum loss of the analyte was obtained at 2.5 min and was used subsequently in the analysis protocol.

The use of the washing and pre-concentration procedures provided a precise means of sample concentration and led to good precision for the quantitative determination. It also assured minimum band broadening by eluting a backflush direction. The removal of by-products extended the life-time of both the guard and the analytical column and avoided overloading of the packing material with constituents of biological fluids, which could cause a loss of column efficiency.

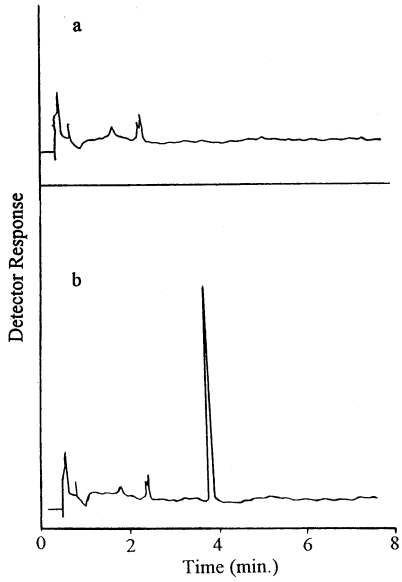

Fig. 3. Chromatographs obtained by analysis of drug-free plasma extract (a) and an extract of plasma spiked with 10 μg ml^{-1} aspirin (b).

Table 1
Linear regression data ($y = mx + b$) and correlation coefficients for plots of aspirin

Day	Equation of the regression line	Correlation coefficient (r)
1	$y = 0.0354x - 0.0022$	0.9984
2	$y = 0.0355x - 0.0012$	0.9994
3	$y = 0.0351 + 0.0022$	0.9978

3.2. Detection of aspirin in human plasma

Typical chromatograms resulting from the analysis of drug-free plasma and drug-spiked plasma by multi-dimensional chromatography are shown in Fig. 3(a–b).

The figure clearly indicates that under the experimental conditions, the drug peak is well resolved from the endogenous plasma peaks flanking it. At a flow rate of 2.0 ml min^{-1}, the determination of aspirin could be performed in ~ 7 min including sampling, washing and separation giving a throughput of approximately eight times per hour. The limit of detection, equal to 0.1 μg of drug per ml of plasma, was calculated according to the three s_d/m criteria, where m is the slope of the calibration graph and s_d is the standard deviation ($n = 5$) of the signals from 0.2 μg ml^{-1}. This limit of detection is in agreement with previously reported values for determination of aspirin in biological fluids [15–18].

The linearity of the method was determined by constructing a linear correlation graph in the range 0.2–12 μg ml^{-1} aspirin using spiked plasma samples, each point corresponding to the average of three injections of the sample. As shown from the data in Table 1, the analysis showed linearity over this range. Using least-squares regression analysis, correlation coefficients greater than 0.996 were obtained over 3 days under the experimental conditions described above.

Precision of the assay evaluated in terms of the relative standard deviation for five replicate injec-

Table 2
Intra- and inter-assay variability

Amount added (μg ml^{-1})	Reproducibility (RSD)%	
	Within-day	Between-day
0.5	1.8	2.3
1	2.4	3.0
3	4.5	5.5
5	5.4	5.8
10	6.0	6.3
Mean	4.02	4.58

Table 3
Recovery studies for control plasma samples spiked with 0, 2, 6, 10, and 12 μg ml^{-1} aspirin

Sample	Aspirin added	Recovery[a]	
	(μg ml^{-1})	(μg ml^{-1})	%
Human plasma	0	0	–
	0.5	0.43	85
	2	1.72	86
	6	5.52	92
	10	9.60	96
	12	11.76	98

[a] Average of three determinations.

Table 4
Interference by various compounds on the analysis of aspirin

Compound	Analyte: Interferent (w: w)	Relative sensitivity (%)[a]
Acetaminophen	1: 1	103
	1: 2	99.5
Caffeine	1: 5	104
Salicylamide	1: 1	105
	1: 10	103
Codeine	1: 1	102
	1: 2	99

[a] Expressed as the ratio of the signal of the analyte in the presence of interferent to that of the analyte alone.

tions of 1 and 2.5 μg ml^{-1} aspirin standard solutions was found to be 2.4 and 4.0%, respectively.

3.3. Reproducibility studies

The reproducibility of the overall method was determined by extracting and injecting seven replicates plasma standards at each of three concentrations, i.e. 0.5,1, 3, 5 and 10 μg ml^{-1}, and by calculating 'the amount of drug found' by interpolation of the y-values (peak height) on the individual regression lines. The values of "amount found" were then used in the calculation of the mean, standard deviation (SD) and coefficient of variations (CV). Both 'between day'- and 'within-day' reproducibilities was assessed for plasma samples as indicated in Table 2. Aliquots of each sample were tested on the same day and the resulting relative standard deviations (RSDs) indicated the within-day reproducibility. Aliquots of the same sample were tested once a day for 1 week and the resulting RSDs. indicated the between-day reproducibility. As shown by the results in Table 2, the method had an overall mean coefficient of variation of 4.02%.

3.4. Recovery studies

The recovery of the extraction method was then estimated by comparing the calculated concentration from extracted standards at different concentration levels, i.e. 0, 0.5, 2, 6, 10, and 12 μg ml^{-1} levels with that of authentic standards, which were injected at the same concentration levels, assuming 100% recovery. The correlation obtained between spiked and measured concentrations was always good. The results in Table 3 show that, at these concentration levels, drug recovery was greater than 85%.

3.5. Selectivity studies

Some commercially available pharmaceutical formulations can contain a combination of pain relievers in addition to aspirin such as, acetaminophen, caffeine, phenacetin, codeine and salicylamide [24]. Other commercial products may contain salicylic acid as an impurity [25]. Table 4 shows the relative sensitivity of the assay in the presence of different species (average of three determinations).

All of the experiments were carried out at 10 μg ml^{-1} aspirin and under the same experimental conditions used for the analysis. The assay response was not significantly affected by the substances tested at levels might exceed their concentrations in the plasma. The procedure was also applied to separate the major metabolic product of the drug in the body from the parent drug, since it is well known that aspirin is hydrolysed in the body to give salicylic acid [26,27]. Fig. 4 shows a representative chromatograms, where a baseline separation was achieved between the drug and its main metabolite product.

3.6. Clinical and pharmacokinetic applications

Previous studies showed that there are differences in drug availability and absorption among different dosage forms of the same drug and these differences are of significant clinical importance [28–31]. The blood (serum or plasma) concentration-time curve is the focal point of such studies and is obtained when serial blood samples taken after drug administration [32]. Plasma level profiles of different commercially available formulations for aspirin were obtained using the described assay method. In all the profiles a post-dosing peak plasma levels followed by a mono-exponential decline of drug concentration with time was observed. Fig. 5 shows that the mean peak concentration (C_{max}) is higher following the soluble preparation compared with that from the disperse formulation. The peak also occurred at an earlier time than following the disperse tablet. The mean elimination half-life ($t_{1/2}$) for the soluble tablet was shorter than that from the disperse form.

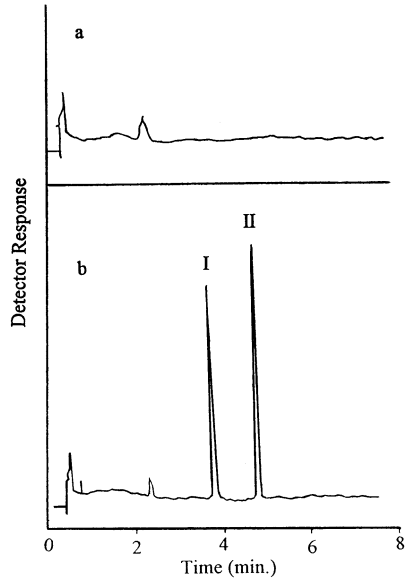

Fig. 4. Sample chromatogram from HPLC assay at 254 nm of drug-free human plasma (A); and a plasma standard mixture (B) containing 10 μg ml^{-1} of aspirin (I) and salicylic acid (II).

In Fig. 6 as expected, the plain aspirin tablet produced significantly higher peak plasma levels of aspirin at an earlier time point than coated aspirin. The plain formulation produced maximum plasma levels, three times faster than the coated tablets. In addition, the plasma aspirin concentration from the plain aspirin was some five times the level achieved by the coated formulation. In general, the enteric-coated formulation gave the lowest levels of aspirin in the plasma, with detectable amounts more than 30 min after ingestion, while in the case of the other formulations a significant quantity of the drug in the plasma was achieved within 10 min. The plasma concentration versus time curve for the soluble form (Fig. 5) can indicate that the soluble formulation presents the aspirin to the gastric mucosa in a form suitable for immediate absorption compared with other formulations which must disperse in the stomach before uptake can begin. The rapid rate of uptake of aspirin from soluble formulation clearly points to the stomach as a major site of absorption.

The calculated peak concentration (C_{max}), the time taken to obtain this level (T_{max}) and elimination half-life ($t_{1/2}$) for the different formulations of aspirin are included in Table 5. C_{max} and T_{max} were obtained from the plasma conc-time curve, the half-time ($t_{1/2}$) values were determined from the elimination rate constant (k_{el}) [33,34] by the equation $t_{1/2} = 0.693k_{el}$. k_{el} was calculated by least squares regression analysis of the data points in the elimination phase of the semilogarthmic plot of aspirin concentrations in plasma versus time curve after oral dose [35,36].

The results described in Table 5 shows differences in the parameters determined, where it can

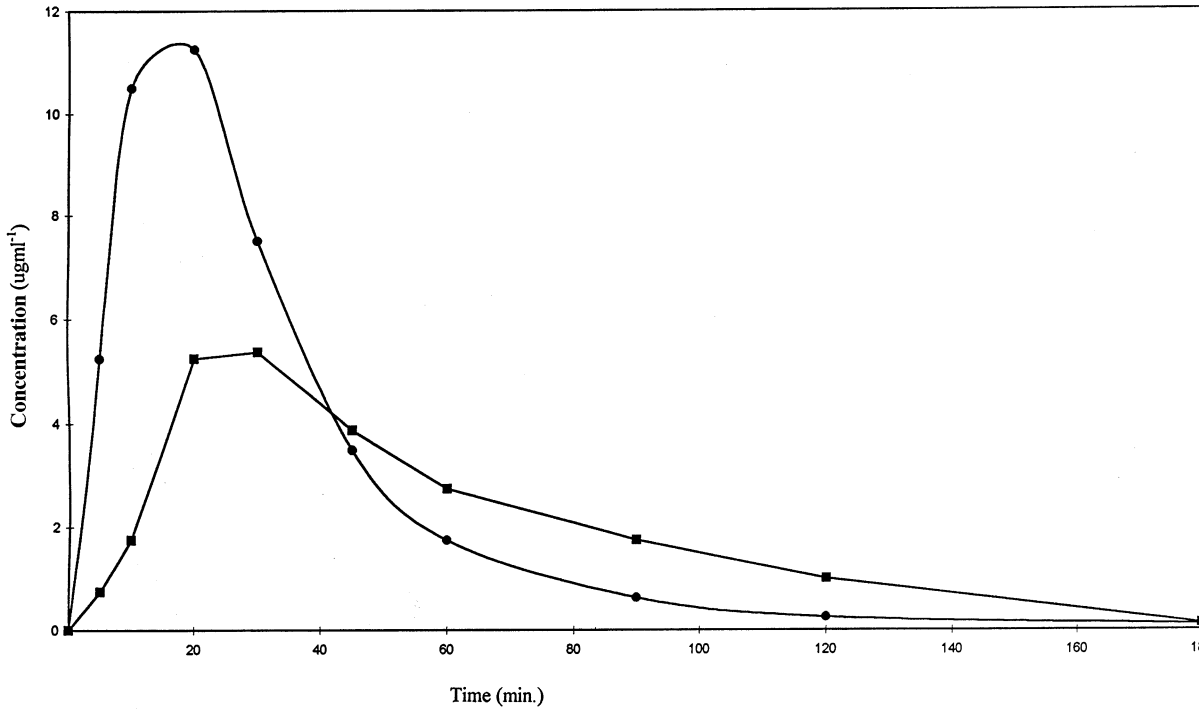

Fig. 5. Plasma concentration time curve from soluble (●) and mouth-dispersible (■) aspirin.

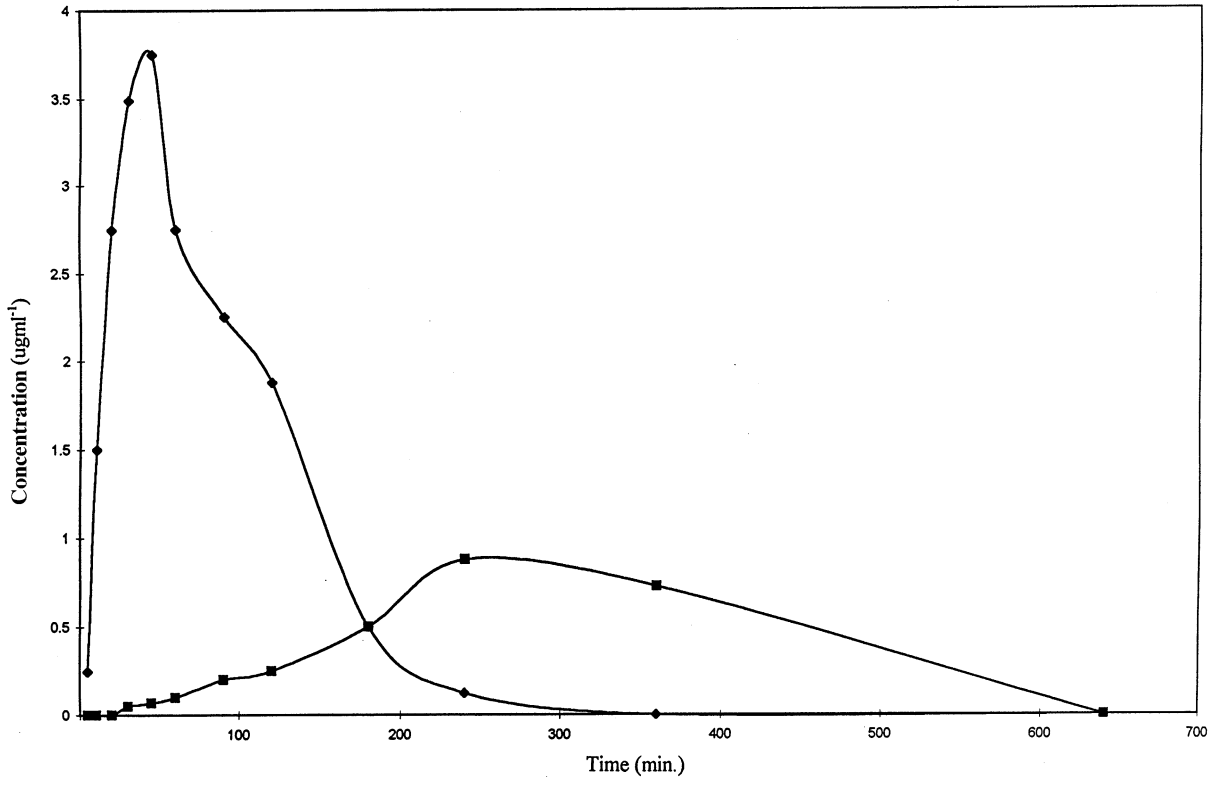

Fig. 6. Plasma level profile of plain (◆) and enteric-coated (■) aspirin.

Table 5
Pharmacokinetic variables for various pharmaceutical formulations of aspirin administered orally

Dosage form	C_{max} (μg ml^{-1})	T_{max} (min)	$t_{1/2}$ (h)	AUC (μg ml^{-1} h^{-1})
Empirin®, plain aspirin (325mg)	3.75	45.5	0.66	6.46
Ecotrin, enteric coated (500 mg)	0.875	240	0.45	3.62
Alka-Seltzer®, effervescent (325 mg)	11.25	20.1	0.32	7.12
Dispirin®, Direct tablets, chewable (300 mg)	5.37	30.5	0.60	5.79

be seen that both soluble aspirin and mouth-dispersible aspirin were absorbed more rapidly than the other dosage forms. These two formulations have shorter T_{max} times than the plain or enteric-coated formulations. Similar variability was observed in areas under the plasma concentration curves (AUC) values, when the different formulations were compared, as presented in Table 5. There appeared to be sizeable, formulation-related differences in AUC, as the average value for the soluble formulation was 44% larger than the enteric coated formulation (6.47 versus 3.62 μg ml^{-1} h^{-1}). In general, the aspirin availability had decreased in the following order: soluble tablets > mouth dispersible tablet > plain tablet > coated tablet. Depending on the obtained results, the soluble form of aspirin should be selected to provide a rapid release, absorption and a therapeutic effect in prevention or treatment of acute myocardial infarction. The enteric-coated form of the drug should be the least favourite for such treatment due to the slow release of the drug from the dosage form.

4. Conclusion

In conclusion, the analytical characteristics of the proposed method and the minimum sample handling required are satisfactory for routine clinical application. Indeed, as no late peak elutes, ~1 h is necessary for the measurement of eight plasma samples. Analysis of plasma samples from healthy volunteers received different formulations of oral aspirin using the method described above, showed a variation in the pharmacokinetic parameters between the different formulations. The study showed that a soluble formulation would have a higher peak plasma levels of aspirin at an earlier time point than the other formulations and it could be the formulation of choice for use in reducing the risk of myocardial infarction. It has recently been recommended that patients judged to be at high risk of myocardial infarction should carry such a product in their pockets for emergency use [37].

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