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Investigation of the metabolism of the neuroleptic drug haloperidol by capillary electrophoresis

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ABSTRACT

Free solution capillary electrophoresis (FSCE) conditions were previously reported to be of limited use for the separation of pharmaceuticals, since many of these compounds are neutral. We show that by consideration of compound hydrophobicity and ionisable functional groups, FSCE conditions can be developed to effect the separation of a drug and its phase I metabolites. This is brought about by adding a suitable organic modifier to aid solubility, and modifying pH to effect a change in the mass to charge ratio of the metabolites present. Furthermore, we show that in this drug metabolism study, FSCE presents an advantage over both reversed-phase HPLC and micellar electrokinetic chromatography. We also demonstrate the use of FSCE for investigation of the phase I metabolites produced by the *in vitro* incubation of haloperidol (a neuroleptic agent) with both mouse and guinea pig hepatic microsomes and show that such an approach can be used to detect both qualitative and quantitative differences in species metabolism.

INTRODUCTION

Haloperidol (HAL) (see Fig. 1) is a clinically proven neuroleptic agent that belongs to the butyrophenone class of drugs used to treat psychotic disorders, such as schizophrenia [1]. It is used in the treatment of hyperexcitable children, as well as in the control of abnormal movements and verbal utterances associated with Tourette's syndrome [1]. However, it has been demonstrated in both humans and animals that the use of this drug can cause debilitating side-effects, such as acute dystonic reactions, akasthisia, tardive dyskinesia, and induction of Parkinsonian-like symptoms [1–3].

Despite widespread clinical use of HAL, little is known about factors that affect correct therapeutic dose and its metabolism. Recently, we and others have investigated the phase I metabolism of HAL using reversed-phase HPLC [4,5], tandem mass spectrometry (MS–MS) [6], and

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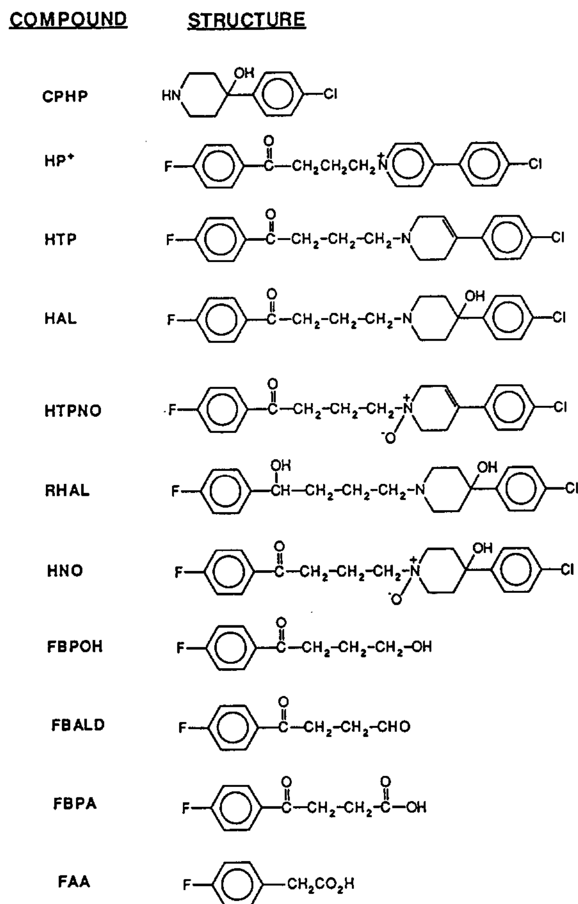

Fig. 1. Structures of the parent drug HAL and ten synthetic standards/putative metabolites.

on-line HPLC–MS–MS [7–11]. These studies have demonstrated that one pathway of HAL metabolism is similar to that involved in the bioactivation of the neurotoxin N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a known Parkinsonian-inducing agent [12–14]. This suggests that a similar mechanism for the induction of Parkinsonian-like symptoms is operating for both compounds [8,10,15].

We are in the process of developing methods to rapidly and efficiently separate such metabolites in order to investigate their pharmacokinetic and toxic properties. Conventional methods for separation of complex drug metabolite mixtures usually involve the use of reversed-phase HPLC. However, phase I metabolism of a drug often results in only minor structural modification of the parent compound, *e.g.*, oxidation, reduction, dehydration, or hydrolysis (see for example ref. 16). These minor structural changes make it particularly difficult to determine suitable chromatographic conditions to effect separation of metabolites due to the limited resolving capability of HPLC. To this end, the high-efficiency separations of capillary electrophoresis (CE) combined with the ease of rapid analysis present obvious advantages over the use of HPLC for the detection of new and possibly relatively short-lived reactive drug metabolites.

The explosive growth of CE in the analysis of biopolymers, such as peptides/proteins, oligosaccharides, and DNA/RNA, has continued unabated [17–19]. However, its use in the analysis of small organic molecules ($M_r < 500$–600), such as conventional pharamaceutical agents, natural products, and drug metabolite products, has received much less attention [18,19].

Most of the reported literature on the use of CE in the separation of small molecules predominantly involves micellar electrokinetic capillary chromatography (MECC). This technique was first introduced by Terabe *et al.* [20,21] and utilizes buffers that contain surfactants at concentrations above their critical micellar concentration (CMC) [22]. Analytes in solution are differentially partitioned between the micellar phase and the surrounding aqueous phase. Since each phase migrates at a different velocity, separation is affected through a variety of complex interactions [23–26]. A number of small molecules, including derivatised amino acids [27], substituted benzenes [28], purine-like bases [29], nucleotides and nucleosides [30], vitamins [25,31–33], and catechols [34–36], have been separated using this methodology. MECC has also been used in the analysis of pharmaceutical agents, such as antibiotics [25,37,38], anti-inflammatory agents [24,25], and antipyretic analgesic preparations [23,25]. Comparative studies that have been undertaken on MECC and free solution capillary electrophoresis (FSCE) in the separation of small molecules have reported that the former is superior in resolving such components [24,26].

The use of CE in drug metabolism studies remains virtually unexplored. Roach *et al.* [39]

used CE to monitor the metabolism of the antifolate methotrexate to its major metabolite, 7-hydroxymethotrexate in serum, and Fugiwara and Honda [40] analysed the levels of the drug γ-oryzanol and its metabolite ferulic acid in plasma. De Bruijn *et al.* [41] have described buffer conditions to separate the pyrimidine analogue 5-fluorouracil and its metabolites by CE. Recently, Johansson *et al.* [42] reported the use of CE coupled to a mass spectrometer (CE–MS) to analyse a mixture of sulfonamide drugs, as well as a series of benzodiazepine drugs.

In the present study, we have investigated the utility of CE in separating metabolites of the neuroleptic drug haloperidol. We have attempted to develop some simple guidelines for buffer selection in order to separate drug metabolites using FSCE. Furthermore, we compare the use of HPLC and MECC with FSCE to separate metabolites of haloperidol. Finally, we demonstrate the usefulness of FSCE in rapidly detecting differences in species metabolism (in this case, guinea pig and mouse) of haloperidol.

EXPERIMENTAL

Chemicals

Haloperidol HAL, 4-(4-chlorophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]-4-piperidinol, potassium phosphate (monobasic), and zinc sulphate were obtained from Sigma (St. Louis, MO, USA). Gold grade ammonium acetate, glacial acetic acid, and magnesium chloride were obtained from Aldrich (Milwaukee, WI, USA). NADP, sodium dodecyl sulphate (SDS), glucose-6-phosphate, and glucose-6-phosphate dehydrogenase were supplied by Boehringer Mannheim Biochemicals (Indianapolis, IN, USA). HPLC-grade solvents methanol and methylene chloride were obtained from Baxter (Minneapolis, MN, USA). High-purity water was prepared in-house using a Sybron Barnstead PCS water purifier system (ex-Millipore) supplied by VWR (Minneapolis, MN, USA). 4-(4-Chlorophenyl)-4-hydroxypiperidine (CPHP), 4-(4-chlorophenyl)-1-[4-(4-fluorophenyl)-4-oxybutyl]-4-piperidinol N-oxide (HNO), 4-(4-chlorophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]-1,2,3,6-tetrahydropyridine (HTP), 4-(4-chlorophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]-1,2,3,6-tetrahydropyridine N-oxide (HTPNO), 4-(4-chlorophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]-pyridinium (HP^+), 4-fluorobenzoyl propionic acid (FBPA), 4-fluorobenzoyl propanol (FBPOH), 4-fluorobenzoyl propanal (FBALD), and 4-fluorophenyl acetic acid (FAA) were synthesised as described previously [11].

High-performance liquid chromatography

The HPLC chromatographic system comprised a ConstaMetric 3000 solvent delivery system (Milton Roy, FL, USA), a Rheodyne 7125 injector with a 100-μl loop and a Rapiscan SA6508 detector set to measure at 220-nm and 245-nm wavelengths. A Tandon TM7002 computer was used to record, store, and analyse chromatograms. Separation was carried out on a 5-μm Hypersil CPS-5 column (250 × 4.6 mm) (Thames Chromatography, Berkshire, UK) coupled with an Upright C-130B guard column (30 × 2 mm) (Upchurch Scientific, WA, USA) packed with 5-μm Hypersil CPS column material. The mobile phase was a combination of acetonitrile (67%) and ammonium acetate buffer (10 m*M*) adjusted to pH 5.4 with acetic acid. The solvent was delivered isocratically at a flow-rate of 1 ml/min [5].

Capillary electrophoresis

Capillary electrophoresis separations were performed using a Beckman P/ACE 2100 Model (Fullerton, CA, USA), coupled to an IBM PS2/76 PC with control and data capture by System Gold software. An uncoated capillary (57 cm × 75 μm) as supplied by Beckman Instruments was used throughout; the effective length of this capillary was 50 cm. Prior to its use, the capillary was rinsed with 0.1 *M* sodium hydroxide (20 column volumes), water (20 column volumes), and buffer (10 column volumes). Between analyses, the capillary was washed with the same order of reagents (10 column volumes of each). The buffer used to afford optimum separation of metabolites was 50 m*M* ammonium acetate containing 10% methanol and 1% glacial acetic acid in water at pH of 4.1. For MECC experiments, a variety of buffers were used, including 50 m*M* phosphate (30 m*M* KH_2PO_4–20 m*M*

Na_2HPO_4), 50 m*M* NH_4OAc, and 100 m*M* borate (sodium borate–boric acid). All buffers contained the surfactant SDS at concentrations above its CMC.

Synthetic standards were individually dissolved in MeOH (1 mg/ml) and 5μl was removed from each vial and mixed in a single, clean vial to give the mixture containing HAL plus CPHP, HP^+, HTP, RHAL, HNO, HTPNO, FBPOH, FBALD, FBPA, and FAA. The mixture was introduced by pressure injection (1 s), and all experiments were conducted with an applied voltage of 30 kV and a capillary temperature maintained at 25°C. Analyte detection was by UV at a wavelength of 214 nm.

Microsomal incubations

English short-hair male guinea pigs were obtained from the Charles River Co. (Montreal, Canada). Animals were fasted overnight before sacrifice. Hepatic microsomal preparations were prepared using the centrifugation method described previously [43].

Mouse hepatic microsomes were prepared by differential centrifugation of freshly prepared liver homogenates from male $CD2F_1$ mice obtained from the National Cancer Institute (Bethesda, MD, USA) [44]. Cytochrome P_{450} enzymes were induced by pretreatment with phenobarbitone (80 mg/(kg day) $\times$ 3 days) prior to sacrifice of the mice on the fourth day [45].

Incubation procedures were as follows: a nicotinamide adenine dinucleotide phosphate (NADPH) generating system consisting of the sodium salt of NADPH ($NADP^+$) (2 μmol), glucose-6-phosphate disodium salt (10 μmol), glucose-6-phosphate dehydrogenase (1 unit), and $MgCl_2$ (2 mg) all in 2 ml phosphate buffer (0.2 M, pH 7.4) was preincubated for 2 min. Enzymatic reactions were initiated by addition of HAL (2 μmol) and microsomal preparations equivalent to 0.5 g original tissue. In control incubates, heat inactivated microsomes were used instead of fresh microsomal preparations. Incubations were carried out for 30 min at 37°C.

Enzymic reactions were terminated by addition of $ZnSO_4$ (200 mg) to the incubation mixture. The precipitated proteins were removed by centrifuging (IEC Cru-5000) at 2300 rpm (*ca.* 1200 *g*) for 20 min. The supernatant was passed through a preconditioned [methanol (4 ml) followed by distilled water (4 ml)] Sep-Pak C_{18} cartridge. Excess $ZnSO_4$ was removed by washing with distilled water (4 ml). The retained compounds were eluted by methanol (4 ml), which was subsequently evaporated to dryness at 45°C under nitrogen [46]. The residues were reconstituted in methanol (200 μl) and subjected to FSCE separation.

RESULTS

High-performance liquid chromatography separation

A mixture consisting of the parent drug HAL and six synthetic standards, previously demonstrated to be metabolites [6], [CPHP, HP^+, HTP, HTPNO, RHAL, and HNO (see Fig. 1)] was used to determine optimal HPLC conditions. IS is the internal standard pirenzepine. In excess of fifty mobile phase solvent systems, as well as a number of different stationary phases, *e.g.*, reversed phase C_{18}, C_4, and phenyl, were also evaluated in order to try to resolve the eight component mixture [5]. Optimum separation conditions were obtained on a 5-μm Hypersil CPS-5 cyano column using an isocratic mobile phase of 67% CH_3CN with 10 m*M* NH_4OAc buffer adjusted to pH 5.4 with acetic acid. The flow-rate used was 1 ml/min [5], and the total run time was 20 min. It should be noted that even using these conditions, it was not possible to baseline resolve the two clusters of com-

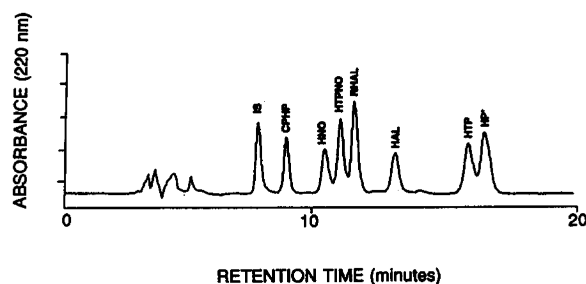

Fig. 2. Reversed-phase HPLC chromatogram (220 nm) of HAL and a mixture of six synthetic standards using a 5-μm Hypersil CPS-5 column, using an isocratic mobile phase of 67% CH_3CN with 10 m*M* NH_4OAc and AcOH (pH 5.4) at a flow-rate of 1 ml/min. IS is the internal standard.

pounds consisting of HNO, HTPNO, RHAL and HTP, HP^+, respectively (see Fig. 2). The detection limit for CPHP and RHAL (monitored at 220 nm) was *ca.* 400 pmol on-column, whereas for the remaining five components (245 nm), it was possible to detect *ca.* 150 pmol of each compound on-column.

Micellar electrokinetic chromatography separation

Previous studies have indicated that MECC is the method of choice for separation of mixtures of small molecules, such as pharmaceutical agents [24,26]. Most workers have reported the use of either phosphate buffer [23,34,35] or borate buffer [47], or a combination of the two [24,26,32,34] in conjunction with SDS to effect MECC separation. In this study, we investigated a variety of buffers containing SDS. We initially used 50 m*M* phosphate [Na_2HPO_4–KH_2PO_4 (1:1)] containing 10 m*M* SDS at pH *ca.* 7.0 in order to effect separtion of a mixture containing *ca.* equimolar amounts of the synthetic standards HNO, HTPNO, FBPOH, and FAA. Only three peaks were detected in the electropherogram (migration times 6.00–6.25 min), and they were not baseline resolved (results not shown). Furthermore, problems were encountered with the solubility of the phosphate buffer in the presence of SDS. Attempts at separating the mixture containing eleven synthetic standards CPHP, HP^+, HTP, HAL, RHAL, HNO, HTPNO, FBPOH, FBALD, FBPA, and FAA (see Fig. 1) using 50 m*M* NH_4OAc with 20 m*M* SDS were also unsuccessful. This in part was due to the high current (*ca.* 210 μA) produced (equivalent to 11 W/m) at 30 kV, leading to substantial Joule heating and arcing in the capillary (results not shown). Partial resolution of the eleven component mixture was achieved using 100 m*M* borate buffer (sodium tetraborate–boric acid) containing 20 m*M* SDS.

Free solution capillary electrophoresis separation

A variety of free solution buffer systems were investigated, including phosphate and borate at various ionic strengths and pH values. However, optimal separation of the eleven synthetic standards was effected in 50 m*M* NH_4OAc containing 1% AcOH and 10% MeOH (pH 4.1), and this is shown in Fig. 3. Electrokinetic separations were conducted using 30 kV across the capillary (57 cm × 75 μm), running a current of 140 μA. Nine of the eleven components were baseline resolved, with only FBPOH and FAA co-migrating as neutrals with the EOF. Under these pH conditions CPHP, HP^+, HTP, HAL, RHAL, HNO, and HTPNO, were of cationic character and migrated before the EOF, whereas FBPA and FAA possessed anionic character and were detected at longer migrations times than the EOF. In all cases, detection limits were *ca.* 100 fmol injected onto the capillary. Since the volume of injection was *ca.* 10 nl, sample concentrations of *ca.* 15 pmol/μl were required to enable component detection. The relatively high current of 140 μA led to some Joule heating which affected the reproducibility of migration times. However, with the exception of the two anionic species FBPA and FAA, relative migration times of the synthetic standards in repetitive analyses were very good. These results are detailed in Table I.

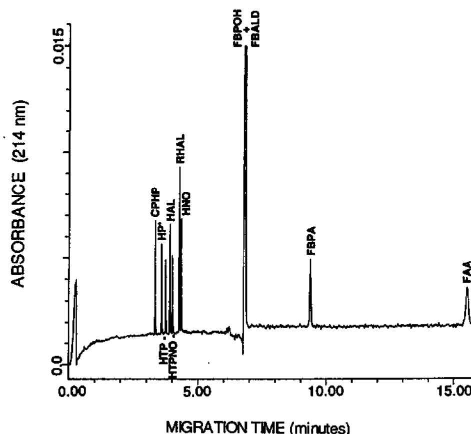

Fig. 3. Electropherogram of HAL and ten synthetic standards by FSCE monitored at a UV of 214 nm. Buffer consisted of 50 m*M* NH_4OAc containing 10% MeOH and 1% AcOH, on a capillary of 57 cm × 75 μm I.D. A voltage of 30 kV gave a current of *ca.* 140 μA.

The mixture of HAL plus the ten synthetic standards was also subjected to FSCE using only 50 m*M* NH_4OAc containing 0.1% AcOH (pH 4.1). Organic modifier (MeOH) was not used in the buffer; however, the sample mixture was

TABLE I

MIGRATION TIMES OF SYNTHETIC STANDARDS RELATIVE TO HAL USING FSCE (50 m*M* NH_4OAc CONTAINING 10% MeOH AND 1% AcOH)

Synthetic standard	Mean migration time relative to haloperidol	R.S.D. (%) ($n = 5$)
CPHP	0.87	0.57
HP^+	0.93	0.43
HTP	0.96	0.00
HAL	1.00	–
RHAL	1.02	0.00
HNO	1.08	0.46
HTPNO	1.10	0.36
FBPOH + FBALD	1.65	1.94
FBPA	2.16	4.49
FAA	3.05	2.82

injected (*ca.* 10 nl) in 100% MeOH. Only five of the eleven components were baseline resolved, namely CPHP, HP^+, HNO, FBPA, and FAA (Fig. 4). Although HTPNO was resolved from the other components, it had very poor peak shape.

Microsomal incubations

Employing conditions developed for the separation of the synthetic standards mixture, both mouse and guinea pig hepatic microsomal incubates were subjected to FSCE. These results are shown in Figs. 5 and 6, respectively. Since microsomal incubates are a complex mixture of proteins and buffers, it was necessary to remove these matrices by precipitation of the microsomal proteins using $ZnSO_4$ and subsequent removal of the $ZnSO_4$ and other salts using a solid-phase C_{18} cartridge prior to analysis by CE.

Comparison of the relative migration times (metabolite to unmetabolised parent drug HAL) of samples with synthetic standards (see Table I) revealed the presence of CPHP, HP^+, HTP,

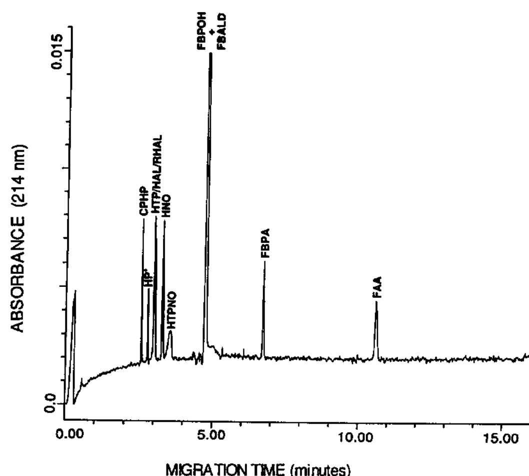

Fig. 4. Electropherogram of HAL and ten synthetic standards by FSCE. All conditions as for Fig. 3 except that the buffer did *not* contain any MeOH.

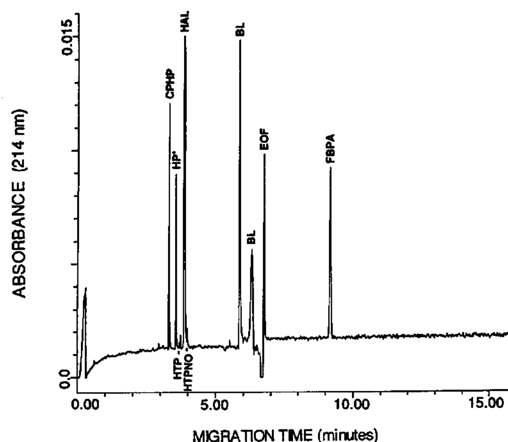

Fig. 5. Electropherogram of a mouse hepatic microsomal incubate. All conditions as described in Fig. 3. BL are observed in a control mouse hepatic microsomal incubate.

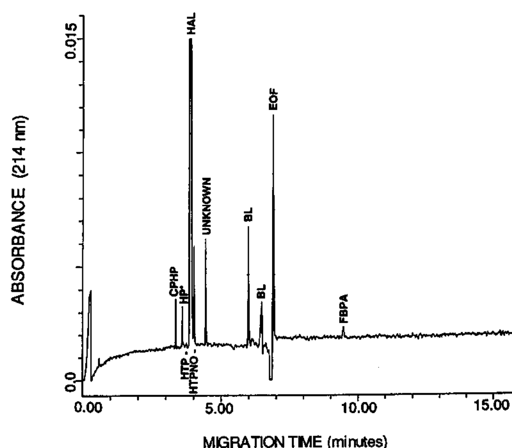

Fig. 6. Electropherogram of a guinea pig microsomal incubate. All conditions as described in Fig. 3. BL are observed in the control incubation. UNKNOWN is a metabolite of HAL yet to be fully characterised.

RHAL, and FBRA in the mouse microsomal incubate (Fig. 5). Analysis of the guinea pig microsomal incubate confirmed the presence of the same metabolites as those found in the mouse incubate, as well as a major new component (marked as UNKNOWN in Fig. 6).

In control incubations, using heat inactivated microsomes, no metabolites of HAL were detected in the electropherograms (results not shown). However, unmetabolised HAL, as well as two unidentified components marked BL in Figs. 5 and 6, were detected.

DISCUSSION

The structural diversity of the multitude of modern drugs available for the treatment of disease is well documented (see for example ref. 48). Hence, the evolution of separation methods for complex mixtures of drug metabolites, derived from both *in vitro* and *in vivo* sources, has tended to be specific for a particular drug or class of drugs. Furthermore, due to the structural similarity of phase I drug metabolites, development of conventional separation techniques, such as HPLC, have tended to be time-consuming and, due to the relatively limited resolving capabilities, difficult to achieve. This is reflected in the HPLC separation of the six synthetic standards of HAL metabolites plus the parent drug shown in Fig. 2. It was not possible to effect baseline resolution of five of the six components even after investigating, in excess of, fifty different solvent systems [5]. In addition, polar molecules, such as FBALD, FBPA, and FAA are often co-eluted with the solvent front in reversed-phase HPLC runs and therefore are difficult to detect.

In the present study, we were interested in determining separation conditions that would be functional for a wide variety of drug types based upon consideration of simple structural features of the drug. Due to the rapid method development and high resolution of components achievable by CE, we have investigated the use of this technology to develop conditions that effect the separation of phase I metabolites derived from HAL. In our studies, both MECC and FSCE conditions were evaluated for this application.

The initial impetus for the development of MECC was to achieve the separation of neutral compounds [49–51], since such species have no electrophoretic mobility and will flow past the detector as a single band under the influence of only the EOF. Under MECC conditions, a variety of factors affect the migration times of analytes, including (a) ion pair formation between solute and micelle; (b) distribution of solutes between micelles and aqueous buffer; and (3) electrophoretic mobility of the solute [26,35]. Thus, while this technique was introduced to separate neutral compounds, it has recently been extended to aid the separation of mixtures of small cations [26,34,35] and anions [26,40]. However, acknowledging the success reported in the literature regarding the use of MECC to separate mixtures of small organic molecules, such as pharmaceuticals [23–25,37,38], *a priori* it is difficult to select suitable buffer conditions to effect separations and a variety of buffer and surfactant compositions have been empirically developed.

Using an empirical approach, we investigated a number of buffers containing SDS to effect the separation of HAL and ten synthetic standards previously determined as metabolites [6–11]. Our most successful results were obtained using a 100 m*M* sodium borate buffer with 20 m*M* SDS. However, under these conditions, still we were unable to baseline resolve all eleven synthetic standards, and co-migration of the majority of analyte species was evident (results not shown).

Due to the complexity of the separation factors affecting MECC, we have been unable to determine optimal buffer and surfactant conditions by consideration of the structures of the synthetic standards. Therefore, we considered the use of FSCE where the separation of component mixtures is dependent on both mass and charge of analytes. Furthermore, we have determined that two simple factors are important for prediction of buffer conditions to effect separation, namely hydrophobic character and ionisable functional groups of analyte molecules, and these factors are discussed below.

The hydrophobicity of a compound is frequently expressed as its partition ratio between

octanol and water [52]. However, a qualitative determination of the hydrophobic character of molecules is readily assignable from consideration of functional groups present. Hence, while aromaticity and aliphatic nature clearly determine the hydrophobic character of the molecule, the presence of quaternary amine nitrogens, polyhydroxyl groups, and carboxylic acid functionality add considerable hydrophilic character to the molecule.

In the consideration of a suitable buffer for FSCE separation of drug metabolites, the solubility of the drug and its metabolities in aqueous buffer systems are dependent upon their hydrophilic character. In the present study, the hydrophobic character of HAL and the synthetic standards of putative metabolites prevented their solubilisation in any of the conventional aqueous buffers used, such as phosphate, borate, or ammonium acetate. However, addition of 10% MeOH was enough to solubilise all eleven components in a 50 m*M* buffer solutions of phosphate, borate, or acetate.

Furthermore, it has been documented that addition of some organic modifiers can dramatically affect resolution of mixtures in both MECC [53] and FSCE [54–56]. It has been shown that addition of MeOH slows EOF due to increased buffer viscosity, leading to improved resolution of analytes [54,56]. This is demonstrated further in the separation of HAL and the ten synthetic standards with and without MeOH in the buffer system. In the absence of MeOH from the buffer (see later for discussion of buffer conditions), only five components of the mixture are baseline resolved (Fig. 4). However, addition of 10% MeOH to the buffer composition slows EOF by approximately 1.5 min and results in a marked improvement of resolution with nine compounds now baseline resolved, as shown in Fig. 3.

It should be noted, however, that addition of certain other modifiers, such as acetonitrile, can decrease EOF but increase electrophoretic mobility [54]. Therefore, selection of an organic modifier to aid in metabolite solubility in the buffer must also include consideration of the effects on EOF and migration times of components, since both will ultimately affect resolution.

A limitation of FSCE is that it cannot separate neutrals, since they possess no electrophoretic mobility. Indeed, some authors have questioned the limitations of FSCE in the analysis of pharamaceutical agents, since most species are neutral [32,57]. However, the majority of pharmaceuticals and their subsequent metabolites possess functional groups that are either acidic (–COOH, –SH) or basic ($-NR_2$). Hence, modifying the pH of the buffer solution affects the charge on the parent drug and its metabolites. In the case of HAL and its metabolites, at acidic pH (4.1) protonation of basic nitrogens led to cationic character for parent drug and several putative metabolites (CPHP, HP^+, HTP, RHAL, HNO and HTPNO). In addition, two reported metabolites, FBPA and FAA, were observed to be relatively strong acids, and hence were predominantly dissociated in buffer solution at pH 4.1 and detected as anions. Only two compounds, FBPOH and FBALD, co-migrated under these conditions, since the functional groups of these species were not ionised at pH 4.1. Hence, both were neutrals migrating with the EOF.

A third consideration for FSCE separations is buffer composition. This is usually developed by empirical investigations [58]. We also investigated empirically the effects of buffer composition on the separation of HAL and its phase I metabolites. The results of these experiments demonstrated that a 50 m*M* ammonium acetate buffer gave better resolution of components (particularly HAL/RHAL and HNO/HTPNO) than a phosphate buffer (1.5:1 mix of KH_2PO_4–Na_2HPO_4) of equal concentration. It is suggested that this is due to the slower EOF developed by the ammonium acetate buffer caused by a lower mobility of the ammonium ion in comparison to that of the smaller alkali metal ions. The mobility of analyte cations is determined by the sum of their electrokinetic mobility and the velocity of EOF [59]. Therefore, better resolution of such species will be achieved with slower EOF, since analytes are on the capillary longer as they migrate slower.

Development of FSCE conditions to separate HAL metabolites was prompted by our interest in the metabolism of this widely used clinical

drug. One area of particular interest is the difference in metabolism by humans and different animals species. Species dependent differences in metabolism can occur in both phase I and II metabolism and can be either qualitative and/or quantitative [60]. Such variations have been ascribed to differences in enzyme activity [16], and more recently, to molecular aspects of gene evolution [61]. Clearly, the problems raised by the variation in metabolism by different species for new and clinically used drugs is important in understanding the toxicological and pharmacological activity of metabolites.

We investigated the *in vitro* metabolism of HAL by both mouse and guinea pig hepatic microsomes using the developed FSCE conditions. The electropherograms resulting from the analysis of the microsomal incubates, after solid-phase clean-up, are shown in Fig. 5 (mouse) and Fig. 6 (guinea pig). Comparison of the relative migration times (metabolite:HAL) of metabolites to standards enable us to tentatively identify five metabolites (CPHP, HP^+, HTP, RHAL, and FBPA) from the mouse microsomal incubate (Fig. 5). The same five metabolites were also tentatively identified in the guinea pig microsomal incubate, as well as a further unknown metabolite (marked as UNKNOWN in Fig. 6). A clear difference in metabolism is exhibited by mouse and guinea pig in both a qualitative and quantitative manner. The induced mouse microsomes produce much more CPHP, HP^+, HTP, and FBPA than produced by the guinea pig microsomes. However, the guinea pig microsomal incubate contains much more of RHAL, as well as the UNKNOWN, as previously described by Fang and Gorrod [5]. Further structural studies are underway to characterise the unknown peak.

CONCLUSIONS

Our studies of haloperidol metabolism have demonstrated that FSCE offers major advantages for separation of this drug and its phase I metabolites over both HPLC and MECC techniques. Furthermore, method development was much faster by FSCE than either of the other techniques. This was also aided by the FSCE mass/charge ratio separation mechanism, since ionisable functional groups can play a role in component resolution at appropriate pH values. Consideration of molecule hydrophobicity and addition of MeOH to the FSCE buffer was also shown to improve component separation.

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