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Ion mobility spectrometry as a high-throughput technique for *in vitro* transdermal Franz diffusion cell experiments of ibuprofen

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ABSTRACT

Rapid, low-cost and sensitive analytical methods are needed to analyse the large number of samples that are generated when investigating the absorption profile of drugs through the skin using Franz diffusion cell experiments (FDC). The goal of this study was to evaluate the potential of ion mobility spectrometry (IMS) for the quantitative analysis of active pharmaceutical ingredients (API) in transdermal research. Ibuprofen was used as a model drug and the optimal IMS parameters were determined using a Doehlert experimental design. To assess the usefulness of the IMS method, FDC experiments using human skin were conducted, covering a concentration range of 0.32–69.57 μg/ml. The resulting analytical samples were analysed using IMS and subsequently compared to HPLC as a reference method. No significant differences were found between the results obtained using both analytical methods, with a mean skin permeability coefficient (K_p) value of 0.013 cm/h. The combination of fast detection times, sensitivity, low costs and easy maintenance of IMS instruments makes this technique an attractive alternative for HPLC in this type of experiments.

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1. Introduction

Over the last decades, transdermal drug delivery has become increasingly popular [1–3]. As a result, the pharmaceutical industry is investing increasing amounts of money and resources on the development of new products that reversibly overcome the skin barrier [4]. Although a new transdermal product is only of value if the clinical pharmacokinetic profile delivers the appropriate pharmacodynamic response needed for the treatment of the patient, preclinical assessments strongly guides the product development. These include *in vitro* experiments for evaluating the penetration of a molecule through the skin or artificial membranes. Moreover, determination of the release rate of the active pharmaceutical ingredient (API) and/or specific excipients from the formulation is not only an important parameter for characterizing its transdermal behaviour, but it can also be considered as a global overall quality attribute which is valuable in the development of a suitable formulation or in the evaluation of possible changes in formulation composition, production parameters and shelf-life stability. Therefore, the regulatory health authorities are generally requesting these diffusion-release tests in the pharmaceutical dossier submitted to obtain the marketing authorisation. Franz diffusion cell (FDC) experiments are emerging as a generally accepted methodology in this field [5]. Franz cells consist of a donor chamber and a receptor chamber, with a membrane (biological or artificial) clamped between both compartments. The product to be examined is brought into the donor chamber, allowing the API to partition into and diffuse through the membrane towards the receptor chamber. At regular time-intervals, a sample is withdrawn from the receptor chamber and the API is assayed to determine the kinetic profile. Typically, this requires minimally 6–12 sampling points over a 24 h period [6]. Moreover, due to the intrinsic variability in the diffusion results obtained, several replicates per condition are required. This results in a large number of samples that need to be analysed. Therefore, rapid, low-cost and sensitive analytical methods are required. Typically, this is done using high-throughput high performance liquid chromatographic (HPLC) methods. Nevertheless, this still remains a time-consuming task and more rapid analytical methods would present a major advantage.

Ion mobility spectrometry (IMS) is in principle such a fast technique. It has been applied in diverse fields, such as the detection of explosives [7–11], screening of chemical warfare agents [12–15], environmental monitoring [16] and screening of illicit drugs [17–19]. Recently, it is also gaining interest for pharmaceutical quality control [20,21], cleaning verification [22,23] and process analytics [24]. In an ion mobility spectrometer, samples are

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introduced, *e.g.* via thermal desorption on a Teflon (PTFE) substrate, and ionized at atmospheric pressure, *e.g.* by an ionization source such as ^{63}Ni. An electric field drives the ions through a drift tube where collisions occur between the ions and neutral buffer gas molecules (*e.g.* purified air). The characteristic speed at which an ion moves under influence of this electric field, *i.e.* its ion mobility, is a distinct characteristic (much like chromatographic k' values) that allows differentiating compounds by size, shape and charge. The IMS can be set to detect either positive or negative ions. The result of the analysis is displayed as a mobility spectrum or plasmagram, which is a plot of peak intensity (for the IONSCAN-LS equipment used this is expressed as digital units, du) versus drift time. The mobility spectrum usually includes a peak for the calibrant, a substance used by the instrument for internal calibration and displayed as a reference point.

The goal of this study was to evaluate the potential of IMS for the quantitative analysis of API-drugs in transdermal FDC experiments. For the purpose of this study ibuprofen was used as a model drug for transdermal research [25]. Different aspects were evaluated: the sample treatment and introduction system was optimized for ibuprofen determination using experimental designs (DOE) in accordance with quality-by-design (QbD) principles.

2. Experimental

2.1. Materials and reagents

Ibuprofen (Ph.Eur grade) was obtained from ABC Chemicals (Vemedia, Wouters-Brakel, Belgium). Phosphate buffered saline (PBS; pH 7.4; 0.01 M), hydrochloric acid and LC–MS grade formic acid were bought from Sigma–Aldrich (Buchs, Switzerland). HPLC grade *n*-hexane came from Sigma–Aldrich (St. Louis, MO, USA). HPLC gradient grade methanol was obtained from Fisher Scientific (Leicestershire, UK). Water was purified using an Arium 611 purification system (Sartorius, Göttingen, Germany) resulting in ultrapure water of 18.2 MΩ × cm quality. Whatman 2 μm PTFE 46.2 mm membranes were purchased from VWR (Leuven, Belgium).

2.2. Skin permeation study

The penetration of ibuprofen through human skin was determined using static FDC (Logan Instruments Corp., New Jersey, USA) with a receptor compartment of 5 ml. Excised human skin from healthy patients that had undergone an abdominoplasty procedure was used. After cleaning the skin with 0.01 M PBS pH 7.4 and removal of the subcutaneous fat, the skin samples were wrapped in aluminum foil and stored at −20 °C for not longer than 3 months. Just before the start of the experiments, the skin samples were thawed and dermatomed to a pre-set thickness of 400 μm using a Padgett model B electrical dermatome (LifeSciences, Plainsboro, USA). The experimentally obtained thickness was determined using a screw micrometer (Mitutoyo, Tokyo, Japan). Skin samples were sandwiched between the donor and the receptor chambers of the diffusion cell. The receptor compartment was filled with PBS, making sure all air under the skin/membrane was removed. The whole assembly was fixed on a magnetic stirrer and the solution in the receptor compartment was continuously stirred using a Teflon coated magnetic stirring bar. Before starting the skin experiments, skin impedance was measured using an automatic micro-processor controlled LCR Impedance Bridge (Tinsley, Croydon, UK) to ensure that there was no skin damage. Skin pieces with an impedance value below 20 kΩ, a validated system suitability cut-off value developed in our laboratory for this type of experiments, were discarded and replaced. Ibuprofen was topically applied to the surface of the skin as 500 μl of an ethanol/water (50:50, V/V) solution (10 mM). The donor compartment was covered with parafilm (American National Can—Pechinney Plastic Packaging, Menasha, WI, USA). The temperature of receptor compartment was kept at 32 ± 2 °C by a water jacket. The available diffusion area was 0.64 cm^2. Samples (200 μl) were drawn at regular time intervals from the sample port (0, 2, 4, 8, 12, 17, 22 and 24 h) and were immediately replaced by 200 μl fresh solution. The analytically determined assay values for the model compounds were correspondingly corrected for the replenishments.

2.3. Ion mobility spectroscopy

2.3.1. Sample treatment

200 μl of each sample obtained from the FDC experiment was transferred into 1 ml amber glass vials, acidified with 20 μl of 0.1 M HCl, and an equal volume of *n*-hexane (*i.e.* 220 μl) was added. The mixture was vortexed for 5 min, subjected to centrifugation at 20,000 × *g* (ambient temperature) and 100 μl of the upper organic phase was removed for IMS analysis.

2.3.2. IMS analysis

IMS analyses were performed using an IONSCAN®-LS (Smiths Detection, Warren, NJ, USA) IM station software (version 5.389) was used for data acquisition and processing. Ibuprofen was analysed in the negative ionization method. The system was equipped with an internal 4-nitrobenzonitrile (4-NBN) calibrant that was injected into the spectrometer with every IMS measurement. A 1 μl sample was deposited onto the Teflon membrane using an autosampler and the volatile solvent was allowed to evaporate. This delay before the data acquisition is programmed as the post-dispense delay and was set to 3 s. The substrate was then introduced into the IMS system and placed on the desorber heater, which was set at a temperature of 100 °C for the final IMS method. Analyte molecules were vaporized and carried from the heated inlet (100 °C in the final method) to the ionization chamber in a flow of dry air as drift gas (300 ml min^{-1}). As the vapors enter the ionization chamber, a 555 MBq ^{63}Ni radioactive source emits low energy β-particles initiating ionization of the analytes. The negative ions were gated into the drift tube. At 25 ms intervals, short 'kick-out' pulses (0.2 ms pulse width) were applied, across the ionization chamber, forcing the ions through an open grid electrode into the ion drift region. The ions were propelled against a counterflow of dry air at ambient pressure towards the collector electrode, where their arrival times and signal intensity were collected. One such step is defined as one scan. In order to increase the signal-to-noise ratio, a number of scans (here: $n = 10$) are co-added before data processing, giving rise to a segment. Within one analysis, several consecutive segments (here: $n = 80$) are recorded. Therefore, the total analysis duration during these experiments was 20 s (*i.e.* 25 ms × 10 × 80). The reduced mobility constant (K_0) of ibuprofen compensates and standardizes for temperature and pressure towards standard conditions as follows:

$$K_0 = \left(\frac{d}{t_d \times E}\right) \times \frac{273}{T} \times \frac{P}{760}$$

where d is the length of drift region in cm, t_d is the time the ion spends travelling the distance d in seconds, E is the applied electric field in V cm^{-1}, T is the buffer gas temperature in Kelvin and P is the pressure in the drift region (in Torr).

In practice, the K_0 of ibuprofen (ibu) (in cm^2 V^{-1} s^{-1}) was calculated with reference to 4-NBN, an internal calibrant that is used to compensate for small changes in barometric pressure and

temperature:

$$K_{0(\text{ibu})} = \frac{t_{d(\text{4-NBN})}}{t_{d(\text{ibu})}} \times K_{0(\text{4-NBN})}$$

where $K_{0(\text{4-NBN})}$ is equal to 1.652 $cm^2\,V^{-1}\,s^{-1}$, a theoretical value that is stated by the instrument's manufacturer.

2.3.3. Experimental design

Based on pilot experiments, an experimental domain was defined over 70–150 °C for both inlet- and desorber temperature and 3–25 s for the post-dispense delay. Optimal settings for these 3 factors were determined using a Doehlert experimental design, a polynomial response surface modelling (RSM) design that allows exploring the relationship between several explanatory variables and one or more response variables [26]. It is a very effective design (Table 1) as it contains only $k^2 + k + 1$ experimental points for k variables. For our 3 variables, a set of minimum 13 experiments is thus required, characterized by the uniform distribution of the experiments in the three-dimensional variable space: 12 experiments are located as a cuboctahedron equidistant from a central point (experiments #1–12), which was repeated 5 times to estimate variability (experiments # 13–17). The design was run 4 times (4 blocks) on different days, *i.e.* each block was run on a different day to estimate the consistency in response behaviour while ambient conditions (*e.g.* pressure and temperature) are different. As part of the development, the following responses (Y) were chosen: cumulative amplitude (in digital units – du) and maximum amplitude (du). The Doehlert design giving the operational conditions to be tested in order to construct the model, was constructed using MODDE 8.0.2.0 (Umetrics AB, Malmö, Sweden). Once the experiments were run and the corresponding response values obtained, the data were analysed in R 2.9.1 (Free Software Foundation Inc., Boston, USA) using linear mixed models for the response, allowing for linear and quadratic effects on inlet temperature, desorber temperature and post-dispense delay as follows:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3$$

where X_1, X_2 and X_3 represent the different variables studied. The significance of the coefficients was evaluated by multiple regression analysis (MRA) based upon the F-test ($P_{<0.05}$, $P_{<0.01}$ and $P_{<0.001}$). Likelihood ratio tests were used for fixed effects and residual likelihood tests for random effects. Heterosedasticity was examined by means of linear mixed models for the log squared residuals from the outcome regression model, allowing for linear effects of inlet (°C), desorber (°C) and runorder, and for random block effects. The parametric bootstrap was used to acknowledge the uncertainty in the estimated residuals in this model.

Table 1
Doehlert design and experiment conditions.

Experiment	Experimental conditions			MaxA (du)
	Inlet (°C)	Desorber (°C)	Post-dispense delay (s)	
1	150	110	14	310
2	130	145	14	255
3	130	122	23	350
4	70	110	14	391
5	90	75	14	369
6	90	98	5	462
7	130	75	14	371
8	130	98	5	446
9	110	133	5	401
10	90	145	14	347
11	90	122	23	374
12	110	87	23	391
13	110	110	14	390
14	110	110	14	398
15	110	110	14	400
16	110	110	14	384
17	110	110	14	387

2.4. Liquid chromatography

A validated HPLC method (Table 3) was used as a reference method for assaying ibuprofen in the receptor fluid. The apparatus consisted of a Waters Alliance 2695 separation module coupled to a Waters 2996 photodiode array detector (DAD) and controlled by Empower 2 software (all Waters, Milford, USA). The analytical column used was a HALO Phenyl-hexyl (50 mm × 4.6 mm, 2.7 μm particle size) column (Advanced Material Technology, Wilmington, USA), maintained at 30 °C. Samples (20 μl) were injected and isocratic elution was achieved at a flow rate of 1 ml/min by a degassed mobile phase consisting of a mixture (35/65, V/V) of 0.1% m/V formic acid in water and 0.1% formic acid in methanol. UV detection was done at 220 nm, with a typical elution time of ibuprofen at 3.7 min.

2.5. Calculation of skin permeation parameters

The cumulative amount of ibuprofen found in the receptor fluid (in μg) was plotted against time (in hours). Linear regression was performed on the steady-state part of the curve. The lag-time as defined by the Organisation for Economic Co-operation and Development (OECD) [2] was calculated from the obtained linear regression curve: by setting $y = 0$, the intercept with the X-axis is obtained. The steady-state flux J_{ss} is the slope calculated from the regression curve of the steady-state linear part, divided by 0.64 to correct for the exposed skin area. From this secondary parameter, the apparent primary parameter K_p (permeability coefficient in cm/h) was calculated according to [27–29]:

$$K_p = \frac{J_{ss}}{C_{dose}},$$

where C_{dose} = applied dose concentration in μg/ml.

3. Results and discussion

3.1. Method development

Ibuprofen, (±)-(R,S)-2-(4-isobutylphenyl)-propionic acid, is a chiral 2-arylpropionic acid derivative used as a non steroidal anti-inflammatory drug (NSAID). The acidic moiety allows this model compound to be efficiently ionized in the negative mode by deprotonation [30].

Because of its relative simplicity and ubiquitous availability, thermal desorption from a Teflon membrane was chosen as sample introduction method. This approach requires the use of an apolar solvent, because polar solvents such as water cannot effectively wet the surface of the Teflon membrane. As *n*-hexane is commonly used for IMS analyses and ibuprofen has adequate solubility in this solvent, we used this solvent for our method development.

Inlet temperature, desorption temperature and post-dispense delay are among the most critical parameters for IMS analysis using the thermal desorption method [15]. In the IONSCAN-LS instrument, the sample tray encompassing the Teflon substrate on which the sample solution was deposited, is moved over to the inlet area, where the desorber heater moves up to seal the inlet. Heat from the desorber (variable one: disorber temperature in centigrades) vaporizes the sample and a flow of carrier gas sweep the vapour through the heated inlet (variable 2: inlet temperature in centigrades) into the ionization region. Before the sample tray is moved to the inlet area, the solvent deposited on the Teflon substrate is given some time to evaporate (variable 3: post-dispense delay in

Table 2
Fixed effects coefficients table.

	Estimate	Std. Error	*t*-value	*P*
(Intercept)	−293.477	94.530	−3.105	0.002
Runorder	3.660	6.069	0.603	0.547
Inlet temperature	6.128	1.187	5.164	0.000
Desorption temperature	10.472	0.970	10.798	0.000
Post-dispense delay	−13.033	1.381	−9.435	0.000
Inlet temperature^2	−0.020	0.004	−4.760	0.000
Runorder^2	−0.229	0.332	−0.689	0.491
Desorption temperature^2	−0.039	0.004	−10.934	0.000
Post-dispense delay^2	0.339	0.048	7.056	0.000
Inlet temperature × Desorption temperature	−0.026	0.005	−5.126	0.000

seconds). To optimize these variables, a Doehlert design was constructed. Although several designs are suited for this objective, the Doehlert design was chosen because of the minimal number of experimental points needed. Moreover, it is extendable in different directions and, if required, new factors can be added to the existing design. The ibuprofen peak height, based on the observed maximum amplitude (MaxA) or cumulative amplitude (CumA) of the mobility spectra, were used as a response. A good correlation between both responses was obtained and as the maximum amplitude is less sensitive to carry-over, this response value was used to built our model. The estimated coefficients and their corresponding standard error for each of the parameters (including second order effects) are given in Table 2. Statistical analysis of the model showed a significant effect for all main factors. From the main effects plot (Fig. 1), the optimal conditions were determined. Inlet and desorption temperature were both set at 100 °C, which is close to the calculated optimal temperature for these variables. The post-dispense delay was kept as short as possible, *i.e.* at 3 s. These experimental values can be rationalized by the ibuprofen melting point of around 80 °C. When the inlet and desorption temperatures are too low, ibuprofen will not evaporate. There is an increase in response with increasing inlet/desorption temperature, until decomposition of the compound. As the hexane evaporates within seconds after deposition of the sample on the Teflon membrane, elongating this step only results in a loss of ibuprofen.

We found mild evidence of an increase in variability with increasing inlet (P = 0.021) and desorber temperatures (P = 0.012). Fig. 2 illustrates the estimated percentage change in standard deviation on the IMS signal obtained with increasing inlet and desorber temperatures. There was no evidence of heteroscedasticity by post-dispense delay.

3.2. Extraction from aqueous samples

For the analysis of ibuprofen samples, the sample introduction into the IMS is performed through thermal desorption on a Teflon membrane. This introduction method necessitates an apolar solvent that can effectively wet the surface of the substrate. However, during FDC experiments, the receptor medium must be compatible with the skin preparation and must assure sufficient solubility of the analyte [6,31]. Therefore, physiological buffers, with or without solubilizers added, are favoured. In our experiments, the Franz cell receptor phase consisted of 0.01 M PBS pH 7.4. Due to the high boiling point of water and the presence of salts, PBS samples cannot be analysed using IMS. This problem was addressed by developing a liquid–liquid extraction method of ibuprofen from the aqueous PBS buffer to *n*-hexane. As ibuprofen is a weak acid (pKa = 4.4) [32], it is almost completely ionized at the physiological pH of 7.4. Therefore, the solubility of ibuprofen in the receptor fluid is sufficient to assure sink conditions during the FDC experiments. When lowering the sample pH to values below 2.4 by adding hydrochloric acid, ibuprofen becomes fully protonated (*i.e.* un-ionized for more than 99%), thereby minimizing the aqueous solubility due to its high hydrophobicity (log$P$ = 3.6). The ibuprofen recovery of this method, determined using validated HPLC analysis, was found to be 87 ± 4% (avg ± std. dev., n = 6). The recovery was found to be reproducible and therefore, the extraction method can be used to determine the quantity of ibuprofen that is present in the FDC receptor phase samples.

3.3. Basic validation of the method

Fig. 3 shows a representative mobility spectrum of an ibuprofen reference using the optimized parameters. A single peak was found for ibuprofen at a drift time of 15.841 ± 0.216 ms (avg ± std. dev., n = 20), corresponding to a reduced ion mobility K_0 of 1.170 ± 3.00 x 10^{-4} $cm^2\,V^{-1}\,s^{-1}$ (avg ± stdev, n = 20). An overview of the validation results for developed IMS versus the reference HPLC method is given in Table 3.

Linearity was studied in the range from 0.25 to 5.00 μg/ml. Twelve concentration points were assayed in quintuplicate. Good linearity was found in range from 0.25 to 1.00 μg/ml. The obtained response obeyed the equation $y = 566.11x - 96.381$ and the squared correlation coefficient (R^2) was 0.9905. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated on the basis of the standard deviation of the response and the slope obtained from the linearity plot. LOD and LOQ were calculated as $3.3\,\alpha/S$ and $10\,\alpha/S$, respectively, where α is the standard deviation of the

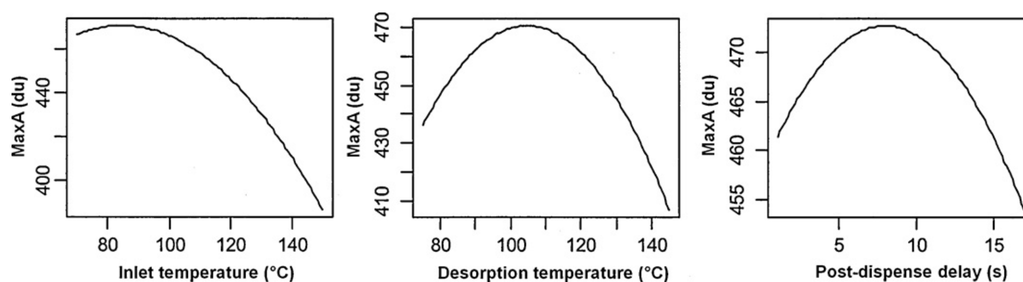

Fig. 1. Main effect plots. The left-panel displays the average response (MaxA in digital units) in function of the inlet temperature (°C), when desorber (°C) and post-dispense delay (s) at their optimal values, and likewise for the other panels.

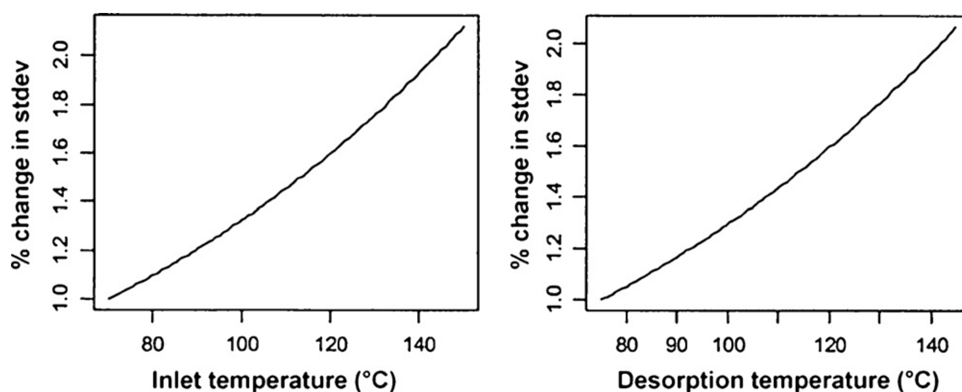

Fig. 2. Estimated percentage change in standard deviation with increasing inlet and desorption temperatures.

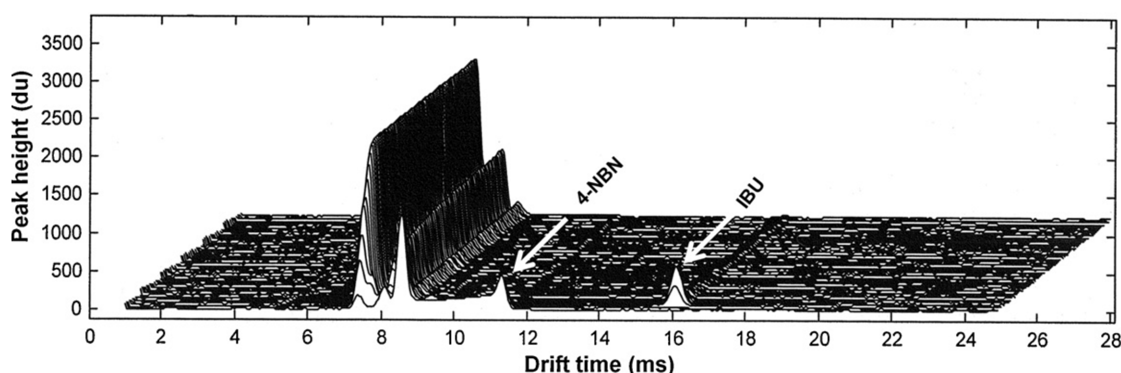

Fig. 3. 3D mobility spectrum obtained using the optimized IMS method (post-dispense delay = 3 s, inlet- and desorber temperature = 100 °C). Ibuprofen can be seen as a single peak with a drift time of around 16 ms. The 4-NBN calibrant drift time is around 11 ms. Chloride peaks, originating from the hexachloroethane reactant used in the negative mode, appear around 7 and 8.2 ms. Due to the presence of oxygen in the drift gas, O_2^- peaks with typical drift times of 8.2 ms are present as well and overlap with the second chloride peak.

Table 3
Validation results.

Parameter	IMS	HPLC
Linearity (R^2)	0.9905	0.9986
Limit of detection (ng/ml)	73	88
Limit of quantification (ng/ml)	222	266
Working range (μg/ml)	0.22–1	0.27–200
Repeatability (RSD, %)	3.03–9.48	0.08–1.63
Accuracy (%)	94.54–96.89	98.94–102.57
Specificity	Interference	No interference

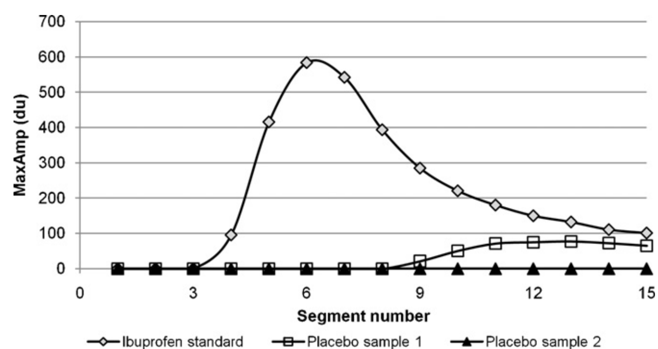

Fig. 4. Desorption profile of ibuprofen standard and placebo skin samples (obtained from two different patients) using the optimized IMS method. The ibuprofen peak attains its maximal amplitude in segments 4–8, while the interfering compound shows maximum amplitude in segments 10–15.

y-intercept and *S* is the slope of regression line. The calculated values of LOD and LOQ were 73 ng/ml and 222 ng/ml, respectively. As can be seen from Table 3, these values are almost identical to the LOQ/LOD obtained using the HPLC method. The repeatability was determined by replicate injections ($n = 5$) of different ibuprofen standards that were in the working range: the relative standard deviation found was 3.03% (1.00 μg/ml), 3.15% (0.50 μg/ml) and up to 9.48% (0.25 μg/ml).

Selectivity of the IMS method was assessed by incubation of skin samples in PBS, ethanol and ethanol/PBS (50:50, V/V) for 24 h. Both the ethanol and ethanol/PBS sample yielded positive interference for some patients: a compound that is extracted from some skin samples is falsely regarded as ibuprofen, due to its identical K_0-value. No interfering peak was observed in HPLC, demonstrating the absence of ibuprofen in those skin samples. The IMS desorption profile of the interfering compound was different from the ibuprofen profile (Fig. 4). A shift is seen in the desorption profile of the placebo sample: while ibuprofen shows maximal amplitude in segment 6, the interfering compound from skin sample 1 elutes at segment 13. The false positive signal can thus be avoided by incorporating the desorption profile in the analytical decision process: the maximal amplitude should lay within segments 4–8. This extra condition increases the selectivity of the IMS method. Other mathematical methods (*e.g.* deconvolution techniques) can be applied as well.

3.4. Permeation study

To assess the usefulness of the IMS method for transdermal research, an *in vitro* human skin flux experimental study was conducted. Ibuprofen was applied to the epidermal side of the skin as 500 μl of a 10 mM aqueous-ethanolic solution. The ibuprofen

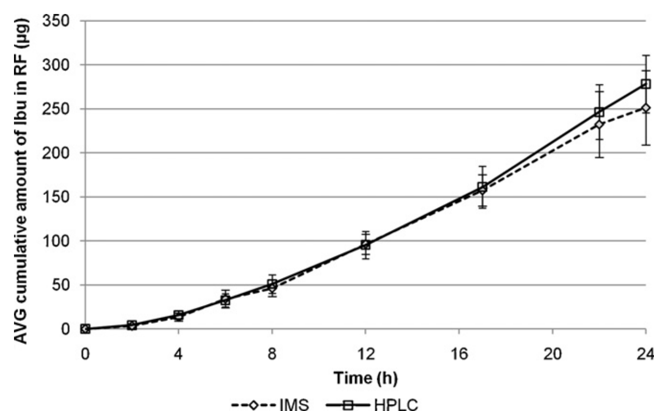

Fig. 5. Mean cumulative amount of ibuprofen (in µg) versus time (in hours) curves in the FDC receptor fluid after application of 500 µl of an ethanol/water (50:50, V/V) solution containing 10 mM ibuprofen (skin diffusional area = 0.64 cm^2, skin thickness = 481 µm).

Table 4
Transdermal parameters calculated using IMS and HPLC data and their difference.

Parameter	Average (n = 5)		Mean difference [95% CI]
	IMS	HPLC	
J_{ss} (µg/cm^2/h)	25.24	21.52	3.72 [−4.78; 12.21]
K_p (cm/h)	0.0142	0.0121	0.00208 [−0.00268; 0.00684]
T_{lag} (h)	4.40	4.50	−0.10026 [−0.75633; 2.99537]

Table 5
Head-to-head comparison of both methods used.

	HPLC	IMS
Analysis time per sample (min)	Up to 20	<1
Sample throughput (per hour)	3	>60
Sample preparation time	Low	Low
Method development	Slow	Fast (due to high sample throughput)
Sensitivity	High	High
Specificity	High	High
Cost per sample	High	Low
Maintenance costs	High	Low
Operator qualification level	High	Low
Solvent waste	Yes	No

concentration in the receiver solution (0.1 M PBS pH 7.4) was determined after extraction via IMS and directly by HPLC as a control. The cumulative amount of ibuprofen that permeated across the skin was calculated and plotted against time. From these curves, it was seen that the amount of ibuprofen that could be detected increased progressively with time (Fig. 5). From the linear part, the steady-state flux J_{ss} (µg/cm^2), the skin permeability coefficient K_p (cm/h) and the lag-time (T_{lag}) were calculated (Table 4). An independent samples t-test revealed that there was no statistical difference (P = 0.05) between the data obtained using IMS or HPLC sample analysis.

Our findings indicate that IMS can be suited for the analysis of FDC samples and in a head-to-head comparison with HPLC, it offers a number of advantages (Table 5). Problems associated with columns, such as poor packing or column fatigue, are non-existent in IMS, thereby eliminating the cost of column materials [20]. Where HPLC analysis of a large number of samples requires large amounts of eluting solvents that need to be discarded as chemical waste, this issue is non-existent with IMS analysis. Moreover, the cost of maintenance of IMS instruments is much lower than that of HPLC [33]. Although both HPLC and IMS are selective and sensitive analytical methods, IMS is generally much faster. Usually HPLC analysis times are between 1 and 20 min [20]. In contrast, the total time from one sample injection to another (*i.e.* including sample disposition, post-dispense delay, data acquisition and processing) for IMS analyses are less than 1 min [22]. This allows quick method development and optimization and increases its competitiveness, especially when a large number of samples needs to be analysed within a short time-frame. In addition, IMS instruments are easy to use, as demonstrated by their widespread employment in security applications, and do not require highly qualified operators. Although our IMS method for the analysis of ibuprofen in FDC samples requires an additional liquid–liquid extraction step compared to the HPLC method employed, this is however often required for HPLC analysis as well (*e.g.* when bovine serum albumin is present in the receptor fluid to assure sink conditions). Therefore, this extra step reflects the variability in sample treatments that can be used. Sample preparation time depends upon the sample form (*e.g.* organic or aqueous), the IMS sample introduction system (*e.g.* Teflon susbstrate or high performance injector) and type of HPLC system (*e.g.* normal phase or reversed phase), but is generally low for both systems.

There are, however, limitations on the use of the IMS technique. The IMS linear range is smaller than those obtained with HPLC. Nevertheless, this was still sufficient for our application, as dilutions can easily be made from the samples so that they fall within the working range. These extra analyses do not greatly influence the total timed needed to analyse FDC samples, seen the short durations and fast analysis time. Moreover, when using a logarithmic standard curve, the dynamic range of the IMS method can be enhanced at least 10-fold. IMS is not capable of independent determination of molecular structure without the use of reference materials or standards for ion mobility [34]. In this respect, it is very similar to HPLC analysis. As IMS equipment can easily be coupled to a variety of mass spectrometers [35], extra separation by mass-to-charge ratio can overcome this issue. In order to enable IMS analysis, compounds must be readily vaporized and ionized to obtain a good signal, and they should be thermally stable [22], similar to gas chromatography.

4. Conclusion

We demonstrated that IMS is a valuable technique in transdermal research using FDC experiments with human skin. After a simple extraction of ibuprofen from the aqueous receptor phase into n-hexane, clear analyte peaks with a reduced ion mobility (K_0) of 1.170 $cm^2\,V^{-1}\,s^{-1}$ within segment 4–8 of the desorption profile were observed. Total analysis times of 20 s were achieved. The combination of fast detection times, selectivity, sensitivity, low costs and easy maintenance of IMS instruments, makes this technique an attractive alternative for HPLC in FDC experiments.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jpba.2011.02.027.

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