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Determination of Pranlukast and Its Metabolites in Human Plasma by LC/MS/MS with PROSPEKT™ On-line Solid-Phase Extraction

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A highly sensitive and selective liquid chromatography/ionspray tandem mass spectrometry (LC/MS/MS) method was developed and validated for the determination of Pranlukast and its oxidative metabolites (SB 240103, SB 241484 and SB 218663) in human plasma in order to support pharmacokinetic studies. The method employed direct injection of human plasma into an on-line solid phase extraction (SPE) PROSPEKT™ instrument for isolation of the analytes followed by column switching to the LC/MS/MS. The use of on-line SPE resulted in reduced sample preparation time and cleaner extracts, therefore minimizing ion suppression and HPLC back-pressures issues. The use of a 20 mM ammonium acetate-methanol system and a step gradient yielded intense ion species, excellent separation between the polar metabolites and the parent drug and sufficient selectivity for baseline resolution of the two positional isomers, SB 240103 and SB 218663. Pranlukast, its metabolites and the internal standard (SK&F 108566) were quantified using a turbo-ionspray interface by negative ion selected reaction monitoring (SRM). The lower limit of quantification (LLQ) for the assay was 10.0 ng ml^{-1} for Pranlukast and 1.00 ng ml^{-1} for its metabolites based on a 100 µl plasma aliquot. The calibration curves were linear for analyte concentrations ranging from 10.0 to 2000 ng ml^{-1} for Pranlukast and 1.00 to 200 ng ml^{-1} for the metabolites. The calculated intra- and inter-assay precision from quality control (QC) samples resulted in mean variability values of less than 12% for all analytes. Pranlukast and its metabolites were shown to be stable under routine analysis conditions for clinical trial samples. The method provides automated sample analysis in a total cycle time of 5 min with improved robustness, sensitivity, selectivity, accuracy and reproducibility compared to the existing methodology. © 1998 John Wiley & Sons, Ltd.

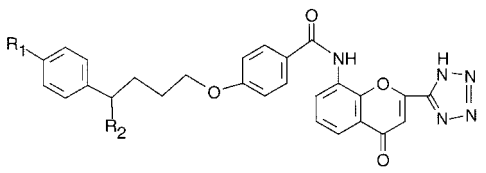
KEYWORDS: liquid chromatography/tandem mass spectrometry; PROSPEKT™; Solid-phase extraction; on line sample preparation; Pranlukast

INTRODUCTION

Pranlukast, (SB 205312, ONO 1078) is a novel potent and selective peptidoleukotriene receptor antagonist which is currently being developed in the USA and Europe for the treatment of asthma. In clinical trials involving asthmatic patients, Pranlukast caused an improvement in pulmonary function as assessed using the forced expiratory volume measurement.[1,2] The chemical structure of Pranlukast is shown in Fig. 1 and its chemical synthesis has been previously reported.[3,4]

Unpublished *in-vitro* hepatic biotransformation studies[5] with Pranlukast in various pre-clinical species have shown that this compound is extensively metabolized to form several oxidative products. Radiolabel metabolic profiling of human plasma indicated the presence of circulating metabolites including the para-hydroxy, benzyl-hydroxy and benzyl-ketone products of Pranlukast[6] (Fig. 1).

The original method for the determination of Pranlukast in human plasma by liquid-liquid extraction, HPLC and ultra-violet (UV) absorbance detection[7]



Compound		R1	R2	M.W.
SB 205312		H	H	481
SB 240103		H	OH	497
SB 241484		H	=O	495
SB 218663		OH	H	497

Figure 1. Structures of Pranlukast (SB 205312) and its oxidative metabolites.

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could not be further developed to include metabolite quantitation due to specificity and selectivity limitations. Off-line extraction procedures (i.e. liquid-liquid and SPE) were slow, labor intensive and not highly reproducible. A later generation of sample preparation utilized semipermeable surface guard columns and column switching devices for on-line SPE and allowed for the quantification of Pranlukast and its three hydroxylated metabolites in human plasma. Although this methodology[8] was exempt from eluant collection, evaporation, reconstitution and sample transfer for injection, it was limited by the repeated use of a pre-column, the dependence of column stability on sample clean-up prior to analysis, occasional column clogging and other reported problems.

With the introduction of the PROSPEKT™ system, automated on-line SPE can be performed on narrow-bore cartridges. Shorter cycle times can be realized due to PROSPEKT's capability for concurrent SPE and LC/MS analysis of the previous sample[9]. Recently, quantitative analysis of drugs utilizing on-line solid phase extraction devices in conjunction with LC/MS/MS[10–13] have generated a great deal of interest due to the speed of analysis, reproducibility, selectivity and sensitivity.

The objective of this study was to establish lower LLQs for the less abundant oxidative metabolites, simplify sample preparation, decrease analysis time, reduce ion-suppressing co-eluants and maximize overall sample throughput. A greatly improved LC/MS/MS method is reported. In conjunction with simple, efficient on-line SPE, this method provided high sensitivity, selectivity and fast sample throughput to quantify Pranlukast and its three hydroxylated metabolites for the analysis of over 6000 human plasma samples.

EXPERIMENTAL

Chemicals

Pranlukast (4-oxo-8-[4-(4-phenylbutoxy) benzoyl-amino]-2-(tetrazol-5-yl)-4H-1-benzopyran hemihydrate) was provided by Synthetic Chemistry, SmithKline Beecham Pharmaceuticals (King of Prussia, PA). Standards of the hydroxylated metabolites of Pranlukast (SB 218663, SB 240103 and SB 241484) were provided by Synthetic Chemistry, SmithKline Beecham Pharmaceuticals (Tonbridge, UK). Internal standard, I.S., (Fig. 2) SK&F 108566 was obtained from Synthetic Chemistry, SmithKline Beecham Pharmaceuticals (King of Prussia, PA). Anhydrous *N*,*N*-dimethylformamide (DMF) was purchased from Aldrich Chemical Co (St. Louis, MO). HPLC grade methanol and acetonitrile were obtained from J. T. Baker Chemical Company (Phillipsburgh, NJ). Reagent grade citric acid monohydrate and ammonium acetate were purchased from Mallinckrodt Chemical Company (Paris, KY). Air, nitrogen and argon were purchased from Praxair, Inc. (Danbury, CT). A Milli-Q Plus Ultra-Pure water system from the Millipore Co. (Bedford, MA) was used to produce HPLC grade water. Control human plasma was obtained from Biological Specialty Corp. (Colmar, PA).

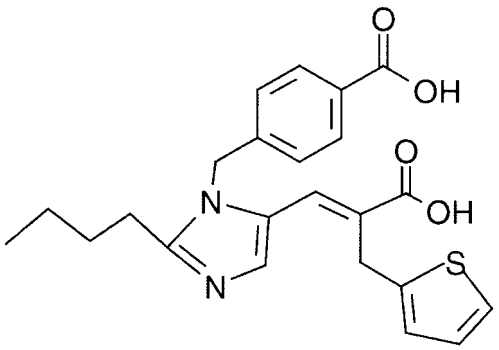

Figure 2. Structure of the internal standard (I.S.), SK&F 108566.

Instrumentation and chromatographic conditions

The PROSPEKT instrument, the Solvent Delivery Unit (SDU) and SPE cartridges (Ph-EC-IST, 10 mm × 2 mm i.d., 40 μm particle size) were purchased from Jones Chromatography (Lakewood, CO). Automated on-line solid phase extraction was performed with the PROSPEKT™ system, which controlled the autosampler, the HPLC pump, the SDU and all switching valves. For any given sample, an appropriate volume of the diluted plasma sample (30–50 μl) was loaded on to the SPE cartridge by a CTC A200SE liquid autosampler (Leap Technologies, Carboro, NC). The sample preparation method for the PROSPEKT™ is detailed in Table 1.

A Flux Rheos quaternary pumping system (Leap Technologies, Carboro, NC) was used to deliver mobile phase to a 30 mm × 2 mm i.d., 3 μm particle size, Hypersil BDS C-18 HPLC column (Keystone Scientific, Bellefonte, PA) which was preceded by a 0.5 μm pre-column filter and a 20 mm × 2 mm i.d. guard column. The HPLC gradient program is listed in Table 2. The HPLC flow rate was set at 300 μl min^{-1}. A post column splitter was set up to allow approximately 60 μl min^{-1} of the column effluent into the MS interface via a fused-silica capillary (50 μm i.d.).

Mass spectrometric detection was carried out using a Perkin-Elmer Sciex API III^+ tandem triple quadrupole system (Concord, Ontario, Canada) coupled to the HPLC via a turbo-ionspray interface. The instrument was operated in the negative ion mode under ionspray MS/MS conditions. The ionspray and interface voltages were −3200 and −650 V; respectively. The orifice voltage −68 V and the collision energy was 23 V. Ultra-pure air was used as nebulizer gas at a pressure of 42 psi and a flow rate of 0.6 l min^{-1}. Ultra-pure nitrogen was delivered as curtain gas at a flow rate of 1.0 l min^{-1}. The auxiliary gas for turbo-ionspray was also made up of ultra-pure air at a flow rate of 5.0 l min^{-1}. The turbo-ionspray probe temperature was set at 350 °C. An equimolar polypropylene glycols mixture in 50% aqueous methanol (1×10^{-4} M) was used for instrument tuning and mass calibration.

The mass spectrometer was operated in the MS/MS mode and data were acquired under SRM. The instrument was set to select the deprotonated molecules

Table 1. Sample preparation program for the PROSPEKT

	SDU			PROSPEKT				
Time (min:sec)	Solvent	Flow-rate (ml min^{-1})	Valve 1 position	Valve 2 position	Valve 3 position	Auxiliary Output 1	2	End time
0:00	H_2O	1	F2 (purge)	F1 (forward)	F1 (online)			
0:20						On (to start autosampler)		
0:21						Off		
0:25		7						
0:40		1						
1:50		7		F2 (back flow)				
2:30		0						
2:35			F1 (elute)				On (start MS acquisition)	
2:36		7		F1 (forward)	F2 (divert)		Off	
3:00					F1 (on line)			
3:20	ACN	7						
4:15			F2 (purge)					
4:30		0						
4:33								End of program

$[M - H]^-$ at m/z 480.0 for Pranlukast, m/z 496.0 for SB 240103, m/z 494.0 for SB 241484, m/z 496.0 for SB 218663 and m/z 423.0 for I.S., by the first quadrupole filter (Q1). The precursor ions were then collided with argon gas at a thickness of 300×10^{13} molecules cm^{-2} in the second quadrupole (Q2) to generate product ions for Pranlukast, SB 240103, SB 241484, SB 218663 and I.S. at m/z 291.0, m/z 172.0, m/z 292.0, m/z 172.0 and m/z 244.0, respectively. Analysis was divided into two periods. During the first period, the precursor-to-product ion reactions being monitored were m/z $496 \rightarrow 172$, $494 \rightarrow 292$, $496 \rightarrow 172$ and $423 \rightarrow 244$ with a dwell time of 90 ms. The transition $480 \rightarrow 291$ for Pranlukast was monitored during the second period with a dwell time of 75 ms.

Routine Acquisition and Display program (RAD, version 2.6, Perkin Elmer Sciex) was used for data acquisition and MacQuan (version 1.4, Perkin Elmer Sciex) was used for the automatic data processing, including integration of chromatographic peaks and fitting of calibration data to a weighted linear regression line.

Preparation of standards and samples

Standard stock solutions (1 mg ml^{-1}) for Pranlukast and its metabolites were prepared in DMF and stored at $-20\,^{\circ}$C. Intermediate stock solutions of Pranlukast and its metabolites (100/10 μg ml^{-1}, 10/1 μg ml^{-1}, 2000/200 ng ml^{-1} and 100/10 ng ml^{-1}) were prepared in DMF with 50% aqueous acetonitrile. Calibration standards were prepared on the day of analysis in duplicate from the intermediate stock solutions for each analytical run at the Pranlukast/metabolites levels of 10.0/1.00, 20.0/2.00, 50.0/4.00, 100.0/10.00, 200.0/20.00, 500.0/50.0, 1000.0/100.00 and 2000.0/200.00 ng ml^{-1}. Both a 1 mg ml^{-1} and intermediate (100 μg ml^{-1}) internal standard stock solutions were prepared in DMF and stored at $-20\,^{\circ}$C. The intermediate stock solution was then used to prepare a 250 ng ml^{-1} working internal standard solution in 1.0 M citric acid.

Prior to extraction, all the plasma samples were vortexed and centrifuged at 3000 g for 10 min. A 100 μl aliquot of the appropriate plasma sample was mixed with 100 μl of the internal standard solution in an autosampler vial (Chromacol, Trumbull, CT). The vials were vortexed for about 15 s after which the samples were ready for injection onto the PROSPEKT SPE system for clean-up and analysis.

Table 2. Gradient program of the HPLC mobile phase

Time (min)	20 mM Ammonium acetate (%)	Methanol (%)
0	75	25
0.3	75	25
0.4	30	70
2.4	30	70
2.5	10	90
3.3	10	90
3.4	75	25

Sample analysis and data processing

Peak-area measurements were obtained for SRM of the specified precursor-product ion transitions. Calibration graphs were constructed by plotting peak area ratios (analyte/I.S.) for standards *vs.* analyte concentrations and fitting the data to a weighted (x^{-1}, x = analyte concentration) linear regression line. Analyte concentration in experimental samples was determined by interpolation from this line.

Method validation

Five pools of plasma QC samples were prepared from stock solutions of all the analytes, which were prepared from a separate weighing, by adding appropriate volumes of stock solutions to drug free plasma. The QC samples were prepared at the expected LLQ, four-times

the LLQ, forty-times the LLQ, one-hundred times the LLQ and the high limit of quantification (HLQ) (10.0, 40.0, 400.0, 1000.0 and 2000.0 ng ml^{-1} for Pranlukast and 1.00, 4.00, 40.00, 100.00 and 200.00 ng ml^{-1} for the metabolites). These plasma samples were stored at $-20\,^{\circ}C$ until analyses were performed. Six replicate samples from each pool were extracted and analyzed on three separate days.

Intra- and inter variability, bias, LLQ and stability were assessed to determine the validity of the bioanalytical method. The mean concentration, standard deviation and the coefficient of variation (CV) were calculated from the set of QC replicates of each concentration and used to determine the intra- and inter-assay precision represented by the between- and within-run precision, respectively. The accuracy was assessed as percent bias. The bias was calculated from the ratio of the mean measured concentration and the nominal concentration multiplied by 100.

Selectivity, the absence of interferences from endogenous components in the biological matrix or exogenous components from the isolation procedure, was assessed by extracting control blank plasma samples from a minimum of six different subjects and also by including control blank plasma samples in each validation run. The lack of interfering peaks at the same analyte retention times was considered as acceptable selectivity.

Stability in plasma

The stability of Pranlukast and its metabolites was determined after 24 h at room temperature and through three freeze-thaw cycles ($-20\,^{\circ}C$ to room temperature). Sufficient volumes of QC samples were prepared for Pranlukast/metabolites at concentrations of 2000/200 and 400/40 ng ml^{-1} to perform each experiment with six replicates. The mean of the peak areas was determined at each concentration for each analyte when fresh and after three freeze-thaw cycles.

Application of the LC/MS/MS method

The disposition of Pranlukast and its metabolites was examined in a healthy volunteer treated with a single oral dose of Pranlukast (300 mg) at FOCUS Drug Development (Neuss, Germany). Arterial blood samples (0.5 ml) were collected at pre-dose, 0.5, 1, 2, 3, 4, 4.5, 5, 5.5, 6, 7, 8, 10, 12 and 14 h post-dose. Plasma was obtained by centrifugation at $4\,^{\circ}C$, packaged, stored frozen at approximately $-20\,^{\circ}C$ and shipped to SmithKline Beecham, King of Prussia, PA, USA. The samples were prepared and analyzed as previously described.

RESULTS AND DISCUSSION

Due to the structural similarity, chromatographic separation of Pranlukast metabolites posed a very challenging task during the method development. It is imperative that sample processing be optimized and that the assay be highly selective and sensitive.

On-line solid phase extraction

Use of the on-line SPE method greatly reduced the sample preparation time and provided cleaner extracts to improve the ionspray MS response due to less ion suppression.[14] A high flow rate of back-flow wash (water, 7 ml min^{-1}) before the cartridge was switched on line with the analytical column seemed to prevent pressure increase of the column, thus improving the robustness of the method. End-capped phenyl chemistry was found to improve analyte recovery, peak shape and adequate retention for rapid analysis compared to other available phases (C-8, C-18, MFC-C-18 and Phenyl).

LC method development

Based on previous chromatographic experience with these compounds, ammonium acetate-methanol and ammonium acetate-acetonitrile systems were evaluated at various pH, ionic strength and compositions. A 20 mM ammonium acetate (pH 6.5)-methanol system was selected for a gradient separation since it yielded the best chromatographic peak shapes for the analytes in plasma and because it promoted the formation of intense deprotonated species. Methanol, which is usually not selected because it leads to higher column back pressures and decreased LC/MS/MS sensitivity,[15] provided the optimal separation of the polar metabolites. The mobile phase system provided sufficient separation selectivity to attain adequate resolution of the benzyl and para-hydroxylated metabolites. Isomer baseline bandwidths were measured accurately at half height. Isomer resolutions of 2.09 ± 0.21 ($N = 9$) and 1.40 ± 0.10 ($N = 9$) were typical at the 2000 ng ml^{-1} and 10 ng ml^{-1} levels, respectively.

The step gradient employed was necessary to elute the non-polar parent compound. The gradient system maintained stable retention times, column pressure and mobile phase composition. In turn, this ensured constant ionization of the analytes which is necessary for quantitation without labeled internal standards, as even the slightest variations contribute to irreproducibility. The analytical column was readily equilibrated between sample injections and the initial cleanup steps. The separation between the parent compound and its metabolites also provided an adequate time change for the acquisition periods allowing for different scan rates between the parent and metabolites. The different scan rates also improved the reproducibility of the assay because of the difference in the LC peak shapes between the polar metabolites and non-polar parent. The retention times of Pranlukast, SB 240103, SB 241484, SB 218663 and I.S. were approximately 3.3, 2.0, 2.2, 2.3 and 1.5 min, respectively, and the injection-to-injection time was 4.55 min.

Mass spectrometric considerations

Each of the analytes was individually infused at a concentration of 5 μg ml^{-1} in 75/25 methanol-20 mM ammonium acetate (pH 6.5). The deprotonated ion for each analyte was identified (Fig. 3) and all Q1 param-

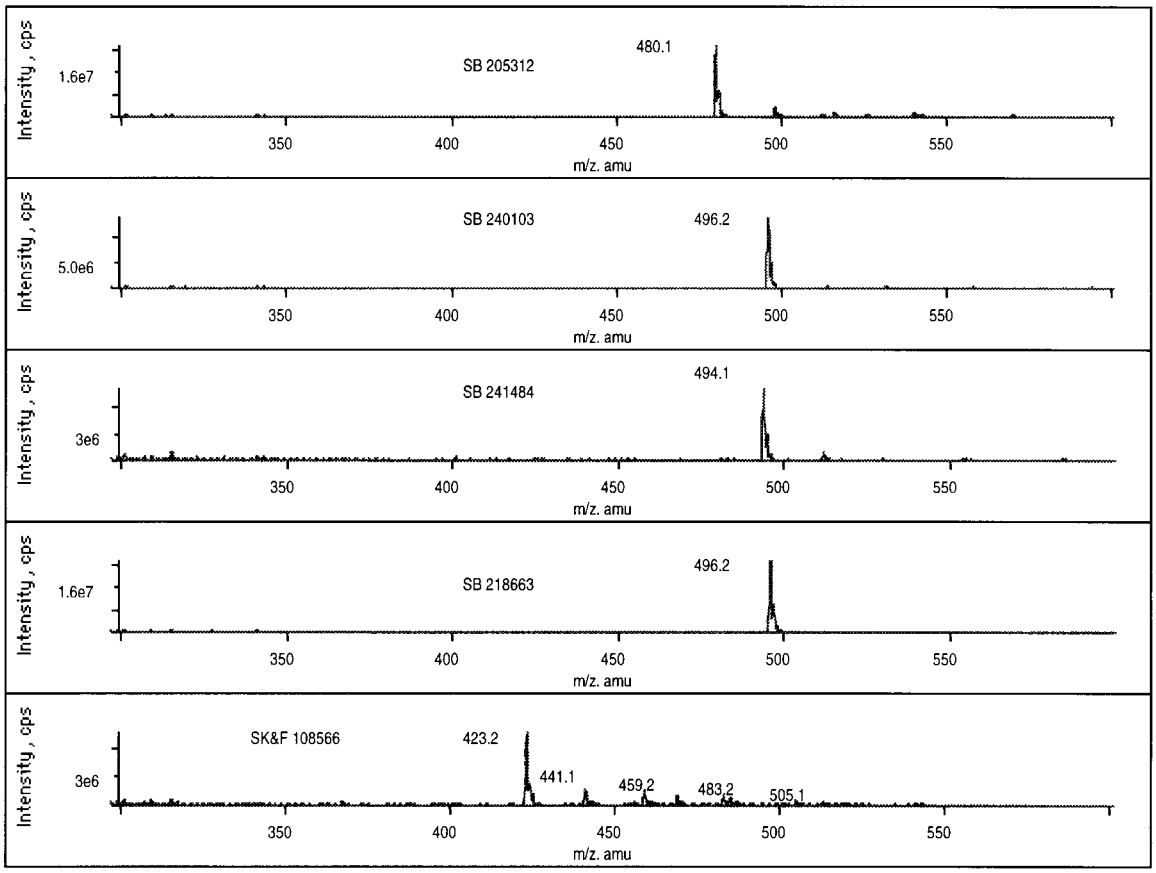

Figure 3. Ionspray mass spectra of SB 205312, SB 240103, SB 241484, SB 218663 and SK&F 108566.

eters were ramped and recorded. Similarly, the product ions produced by CID were identified and their sensitivities were maximized by ramping of the parameters. Due to the nature of the Sciex API III and software, it was not possible to use the optimum parameters for the individual components when analyzing multiple components. It was therefore necessary to compromise the parameters, giving most consideration to the least sensitive component. The parameters were then saved into one file. Each analyte was again checked against the compromised state file to provide adequate sensitivity and reproducibility. The deprotonated ions at m/z 480, 496, 494, 496 and 423 for Pranlukast, SB 240103, SB 241484, SB 218663 and I.S., respectively are of base intensities. The ionization process was significantly increased by the use of turbo-ionspray and was optimal at a temperature of 350 °C.

As illustrated in Fig. 5, CID of Pranlukast and its metabolites is predominantly characterized by the loss of the tetrazole nitrogens, the loss of the alkyl phenyl functionality and amide bond cleavage. MS/MS detection in SRM mode was used with the transitions of $480 \rightarrow 291$ for Pranlukast, $496 \rightarrow 172$ for SB 240103, $494 \rightarrow 292$ for SB 241484, $496 \rightarrow 172$ for SB 218663 and $423 \rightarrow 244$ for I.S. The product negative ion ionspray mass spectra of Pranlukast, its metabolites and internal standard are presented in Fig. 5.

Electrospray ionization of Pranlukast (Fig. 3) was found to produce the single charged species $[M - H]^{-1}$ (m/z 480). The product ion spectra of the precursor ion produced by CID shows highly abundant product ions (Fig. 5). The tetrazole group loss produced ions at m/z 452 and m/z 424 corresponding to the ejection of N_2 and 2 N_2, respectively. The loss of the alkyl phenyl group yields an m/z 291 ion which is presumably due to a homolytic bond cleavage. A similar process seems to be responsible for the amide bond cleavage that yields an m/z 171 fragment ion (Fig. 4).

It was noticed that Plasma concentration range of Pranlukast can be 100 fold higher than that of some metabolites. In order to simultaneously quantify Pranlukast and metabolites in one analytical run, assay sensitivity needed to be adjusted to avoid signal saturation for Pranlukast at high concentration and to enable low quantification limit for metabolites. The product ion 291 for Pranlukast was chosen for SRM detection in the second acquisition period, when count-by-ten mode was utilized. This effectively reduced sensitivity to Pranlukast by 10 fold and further compensated for the sensitivity differences between the parent compound and its metabolites.

Electrospray ionization of the oxidative metabolites yielded single charged species $[M - H]^{-1}$ (Fig. 3). The para-hydroxylation of Pranlukast's phenyl moiety (i.e. SB 218663) yielded a structure which favored heterolytic bond cleavage resulting in even electron ions at m/z 292 and 172 corresponding to the loss of the alkyl phenyl group and the amide bond cleavage, respectively. The incorporation of a hydroxyl group in the benzyl position (i.e. SB 240103) produced a CID spectrum similar to that of its positional isomer with characteristic heterolytic bond cleavage and formation of ions at m/z 468,

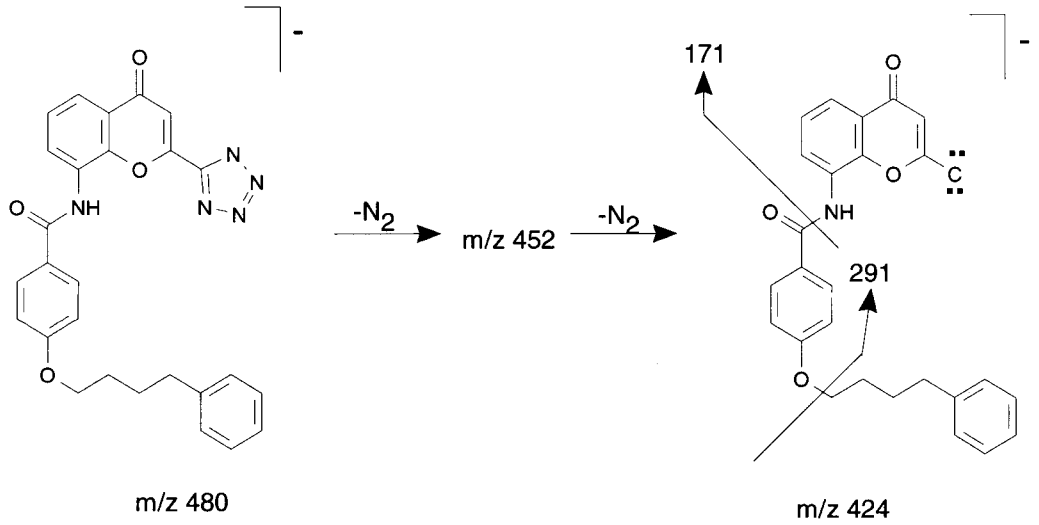

Figure 4. Major fragmentation modes observed upon collisional activation of SB 205312.

440, 292 and 172. The benzyl ketone metabolite, SB 241484, also exhibits the diagnostic ions at *m/z* 466 and 438 and heterolytic cleavage ions at *m/z* 292 and 172. In order to maximize the sensitivity of the metabolites, count-by-one mode was used in the first acquisition period. The product ion spectra of the precursor ions produced by CID are shown in Fig. 5.

Assay characteristics

Sensitivity. The proposed LLQs for Pranlukast and its metabolites were validated by the analysis of six QC replicates on three different runs. The LLQ's for Pranlukast and its metabolites were 10.0 and 1.00 ng ml^{-1},

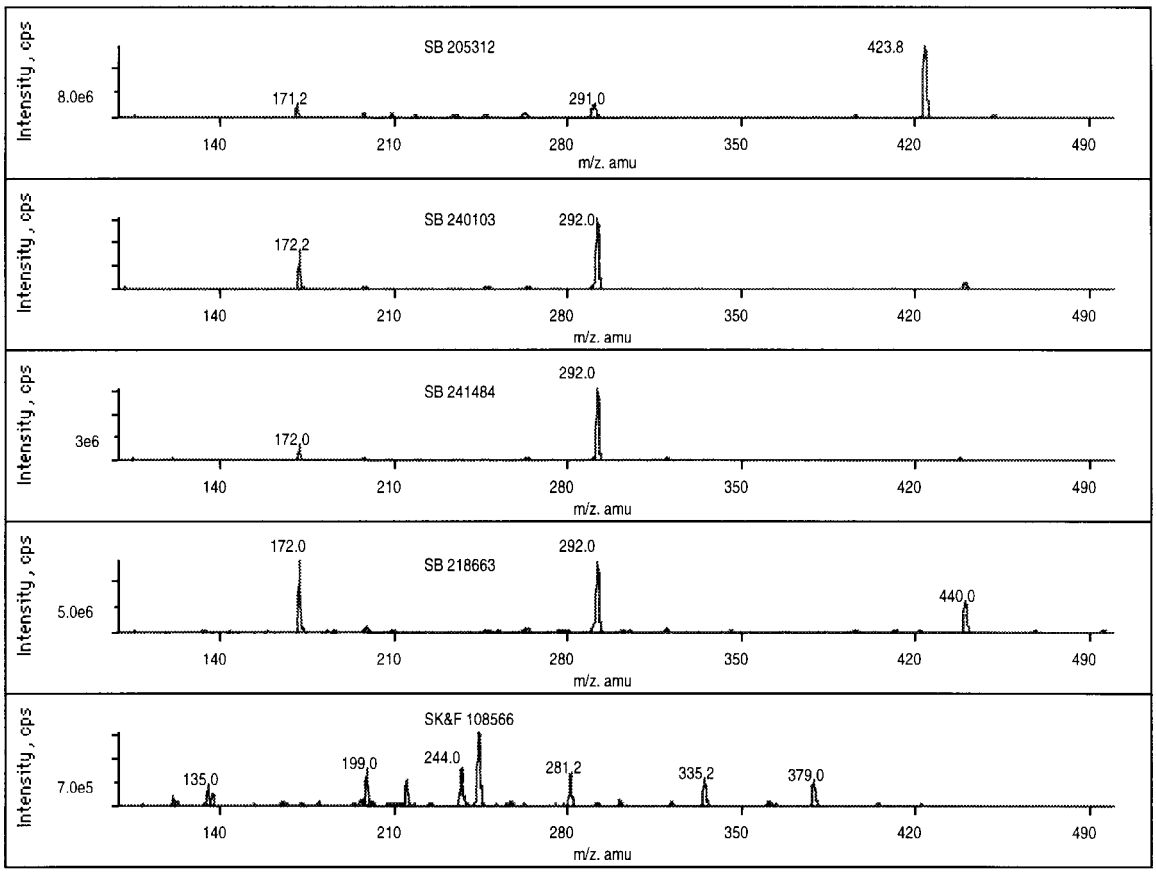

Figure 5. Product ion spectra of SB 205312, SB 240103, SB 241484, SB 218663 and SK&F 108566.

Table 3. Summary of precision and accuracy from the QC samples

Compound		Nominal concentration (ng ml^{-1})				
		10	40	400	1000	2000
SB 205312	Average within-run precision (%)	6.41	8.40	6.53	6.50	3.75
	Between-run precision (%)	10.10	0.40	4.26	1.87	1.89
	Average bias (%)	−7.46	−0.20	1.18	0.91	−1.09
		1	4	40	100	200
SB 240103	Average within-run precision (%)	7.35	5.33	6.51	3.73	5.24
	Between-run precision (%)	5.45	8.05	6.26	4.16	4.13
	Average bias (%)	0.19	4.80	7.50	7.59	3.85
		1	4	40	100	200
SB 241484	Average within-run precision (%)	5.60	5.54	5.95	3.36	4.56
	Between-run precision (%)	11.83	1.19	1.22	4.53	2.86
	Average bias (%)	−4.08	−2.99	1.57	4.01	1.84
		1	4	40	100	200
SB 218663	Average within-run precision (%)	8.05	7.13	6.72	5.40	4.83
	Between-run precision (%)	7.87	1.15	2.57	3.48	0.66
	Average bias (%)	−0.58	−2.35	−0.72	1.25	4.40

respectively for an aliquot of 100 μl of plasma. The CV's for these concentrations were less than 4% for Pranlukast and less than 7% for its metabolites.

Precision and accuracy. Table 3 summarizes the intra- and inter-assay precision and inter-assay accuracy for Pranlukast and its metabolites from the QC replicates. The intra-assay precision was less than 9% for each QC level of Pranlukast and its metabolites. The inter-assay precision was less than 11% for each QC level of Pranlukast and less than or equal to 12% for each QC level of its metabolites. The average inter-assay accuracy, also determined from the QC samples, was within ±8% for each QC level of Pranlukast and its metabolites.

Linearity and selectivity. Linearity was observed for Pranlukast and its metabolites at ranges of 10.0–2000.0 ng ml^{-1} and 1.00–200.00 ng ml^{-1}, respectively. The data were fitted using x^{-1} weighting and resulted in correlation coefficients better than 0.996. Table 4 summarizes the slope, intercept and R^2 values for three validation analyses. Based on the analysis of drug free plasma from six different control plasma lots and blank samples included in each validation run, endogenous or chemical components did not interfere with the drug and internal standards over the concentration ranges described herein.

Stability. The percent differences of the mean calculated concentration of the samples after three cycles of freezing and thawing were within ±9, 9 and 13% for the metabolites, Pranlukast and I.S., respectively. The percent differences of the mean calculated concentration of the samples stored for 24 h at room temperature compared to the 0 h samples were within 19, 11 and 2% for the metabolites, Pranlukast and I.S., respectively. This information provides confidence to perform repeat analysis for clinical samples within 24 h.

Clinical applicability

The method was applied to determine plasma concentrations of parent drug and metabolites for clinical samples. Following a single oral dose of 300 mg Pranlukast as a tablet (wet-granulation), the maximum concentrations achieved for Pranlukast, SB 240103, SB 241484,

Table 4. Summary of slope and intercept data from the calibration curves

Compound		Run 1	Run 2	Run 3
SB 205312	Intercept	0.018 03	0.010 23	−0.002 35
	Slope	0.007 44	0.008 15	0.011 85
	Correlation coefficient	0.998 62	0.998 25	0.999 03
SB 240103	Intercept	0.006 51	0.000 65	−0.005 94
	Slope	0.039 36	0.036 48	0.070 88
	Correlation coefficient	0.999 23	0.998 29	0.997 65
SB 241484	Intercept	0.023 87	−0.011 28	−0.022 41
	Slope	0.107 47	0.107 58	0.165 95
	Correlation coefficient	0.998 92	0.998 67	0.996 25
SB 218663	Intercept	0.005 61	−0.013 08	−0.010 48
	Slope	0.057 23	0.059 84	0.086 19
	Correlation coefficient	0.999 09	0.997 48	0.998 04

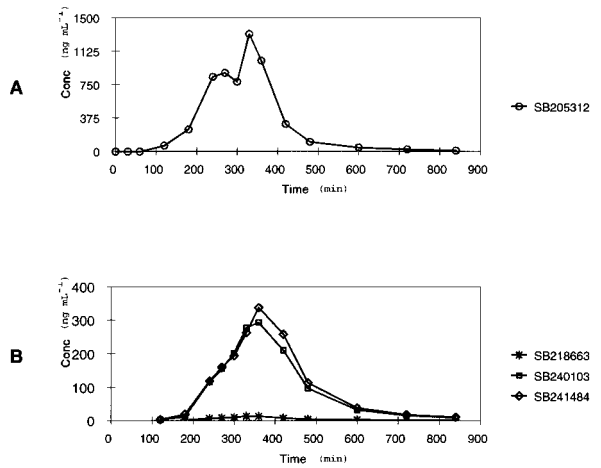

Figure 6. Plasma concentrations *vs*. time curves from a representative volunteer depicting (A) parent drug absorption and (B) metabolite absorption. The subject received a single dose of Pranlukast.

SB 218663 were 1.32×10^3, 293, 337 and 12.9 ng ml^{-1}, respectively. Figure 6 depicts a representative plasma concentration-time profile for Pranlukast and its metabolites. As reported and described in a previous study,[16] double peaking is observed for the parent during the period of maximal absorption. A typical chromatogram for a sample representing a timepoint within this profile is shown in Fig. 7. Primary and secondary pharmacokinetic parameters for each analyte can be readily determined using non-compartmental analysis.

CONCLUSIONS

A LC/MS/MS method for the simultaneous determination of Pranlukast and its three oxidative metabolites in human plasma has been developed, validated and applied to clinical samples. Not only does this methodology have direct applicability to simultaneously measure drugs and their metabolites, but also to determine multiple structurally diverse compounds as is found in discovery screening studies. Sample preparation is simple and is not labor intensive since there is no need for off-line SPE extraction or other sample cleanup. Cross-contamination, pressure buildup and occasional line clogging have been eliminated by the PROSPEKT™ system. This LC/MS/MS method has been demonstrated to be sensitive, accurate, selective and reproducible. Finally, the highly increased sample throughput has already been proven to meet the requirements of the ongoing pharmacokinetic investigation of Pranlukast.

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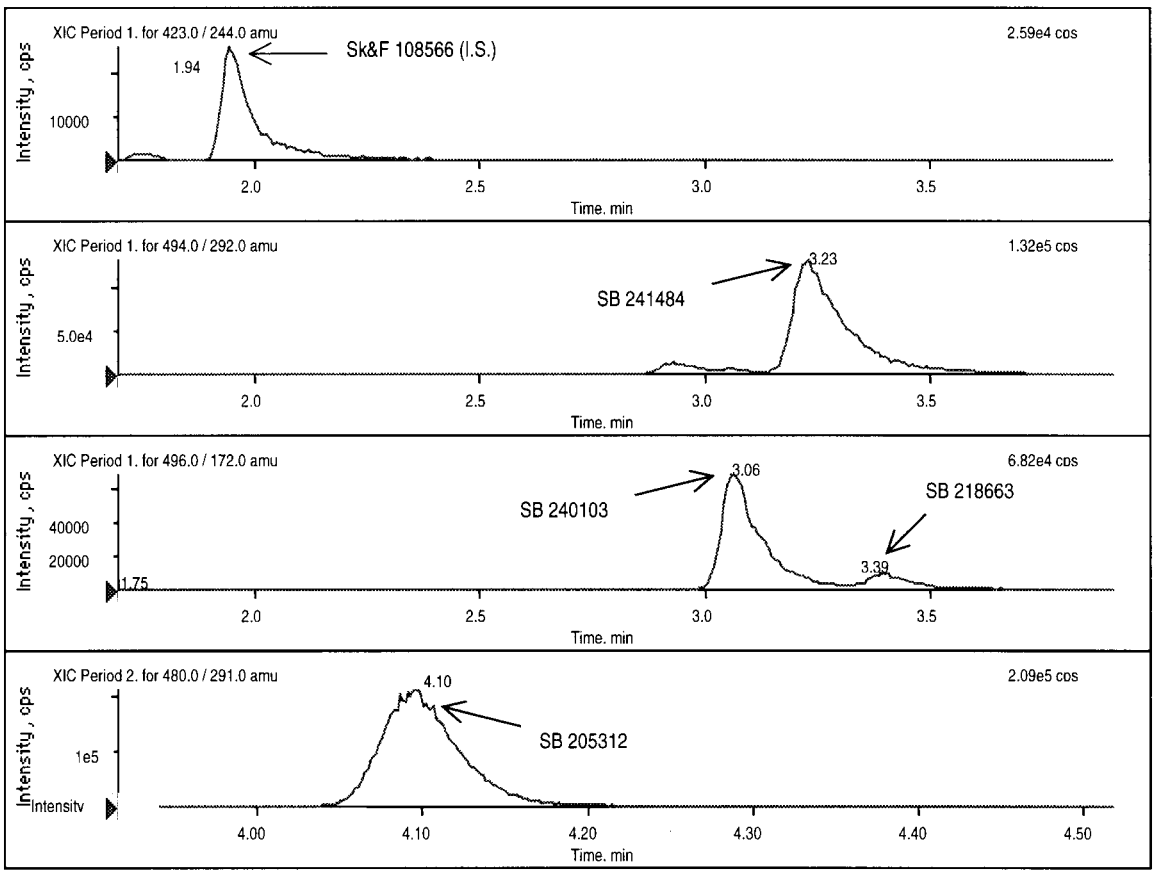

Figure 7. Typical chromatogram of sample time point within the concentration–time profile.

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