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QUANTIFICATION OF IBUPROFEN AND PSEUDOEPHEDRINE IN HUMAN PLASMA BY LC/MS/MS FOR PHARMACOKINETIC STUDIES

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ABSTRACT. A simple, fast and sensitive LC/MS/MS method for quantification of ibuprofen and pseudoephedrine in human plasma has been developed and validated. The lower limit of quantification was established at 0.05 µg/ml IBU and 5 ng/ml PSE. Ibuprofen and pseudoephedrine were separated under isocratic conditions on a Zorbax RX C18 column (3.0 x 100mm, 3µ).

Keywords: ibuprofen, pseudoephedrine, LC/MS/MS, bioequivalence

INTRODUCTION

Ibuprofen (IBU), 2-(4-isobutylphenyl)propanoic acid (Figure 1), is a non-steroidal anti-inflammatory drug commonly used as racemic in treatment of pain and inflammation, available in a variety of preparations [1].

Pseudoephedrine (PSE), (1*S*, 2*S*)-2-(methylamino)-1-phenylpropan-1-ol, is a decongestant that relieves the symptoms of nasal and sinus congestion [2].

Products containing IBU and PSE provide decongestant, antipyretic, and analgesic properties [3,4].

IBU is approximately 80% absorbed from the gut; the suspended form is absorbed at about twice the rate of the tablet form. Although absorption is slower if the drug is taken with food, the extent of absorption is not affected. Peak serum concentrations are reached 1—2 hours after a dose. IBU is highly protein-bound (about 90—99%) and undergoes biotransformation in the liver. Plasma half-life is between 2 and 4 hours. IBU is excreted in the urine, 80—90% as metabolites and approximately 10% as unchanged drug. Excretion is usually complete within 24 hours of oral administration.

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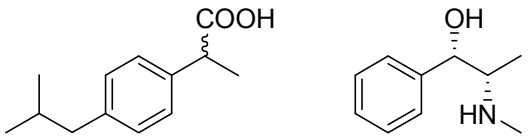
PSE is generally well-absorbed, and its absorption is not affected by food. PSE is presumed to cross the placenta, blood brain barrier, and may be distributed into breast milk. PSE is incompletely metabolized in the liver to norpseudoephedrine, the primary active metabolite. The drug and metabolite are excreted in the urine, with 55—75% excreted as unchanged drug. The elimination half-life of the drug ranges from 6—15 hours dependent primarily upon urinary pH. The rate of urinary excretion is accelerated upon urinary acidification to a pH near 5. Upon alkalinization of the urine to a pH of approximately 8, some of the drug is reabsorbed into the kidney tubule and the rate of urinary excretion is slowed.

Several instrumental methods have been proposed for the determination of either IBU or PSE in biological fluid, including high performance liquid chromatography, liquid chromatography coupled with mass spectrometry. Mainly, high performance liquid chromatography (HPLC) methods with UV detection were described [5, 6]. Liquid chromatography coupled with mass spectrometry (LC/MS or LC/MS/MS) methods were reported also [7, 8]. LC/MS has been widely accepted as the most used method in the identification and quantitative analysis of drugs and its metabolites because of its superior sensitivity and specificity. In the same time, several instrumental methods have been proposed for determination of pseudoephedrine in biological fluids, either individual, either in combination with another drug than ibuprofen [9, 10].

For simultaneous determination of both analytes in biological matrix, only little information was available. For determination of both in pharmaceutical combinations few methods based on spectrophotometric determination, using ratio spectra derivative and multivariate calibration techniques were found [11].



IBU **PSE**

Figure 1. Structure of racemic ibuprofen (IBU) and pseudoephedrine (PSE)

The aim of present study is the development of a rapid and specific LC/MS/MS method for the quantification of IBU and PSE in human plasma in order to be applied in pharmacokinetic and bioequivalence studies. In comparison with previously published HPLC methods, the proposed method has the advantage of quantification of both analytes in a single run.

RESULTS AND DISCUSSION

In the electric field created by the electrospray ionization source, the molecule of IBU readily loose a proton, a negative ion being generated, with m/z 205. Thus, the detection of IBU was carried out in multiple reaction monitoring (MRM) mode, by fragmentation of the parent ion with the m/z 205. The fragmentation of the parent ion is induced by collision of the ion with helium in the ion trap and stable product ions with m/z 159 and m/z161 are observed in the mass spectra (Figure 2). Finally, the sum of abundance of ions with m/z 159 and m/z 161 was monitored and quantitatively correlated with IBU concentration. The retention time of IBU was 4.7 min (Figure 5) and, due detection specificity, no significant interference was observed at the retention time in plasma blank samples chromatograms.

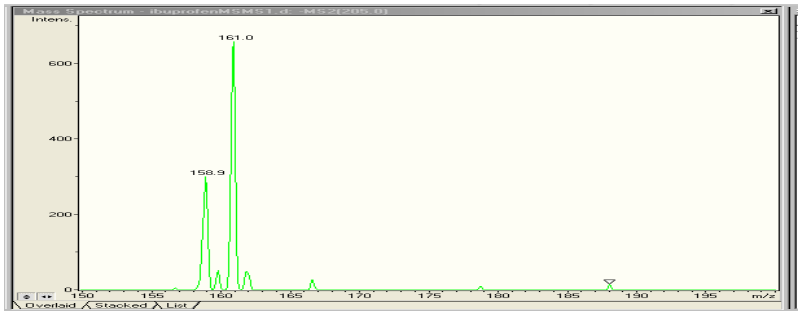


Figure 2. MS/MS spectra of IBU

In the same time, an intense pseudo molecular ion with m/z 166 was observed for PSE after positive ionization of the analyte. The fragmentation of the parent ion is induced by collision of the ion with helium in the ion trap and stable product ion with m/z 148 is observed in the mass spectra (Figure 3). Finally, the abundance of ions with m/z 148 was monitored and quantitatively correlated with pseudoephedrine concentration. The retention time of PSE was 5.3 min (Figure 5) and no significant matrix interference was observed at the retention time in plasma blank samples chromatograms.

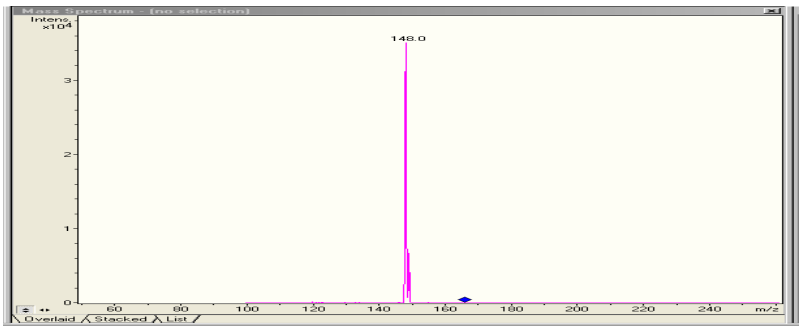


Figure 3. MS/MS spectra of PSE

Sodium diclofenac (DCL) was used as internal standard, the MS transition monitored in its case being m/z 250 > m/z 293, in positive mode (Figure 4).

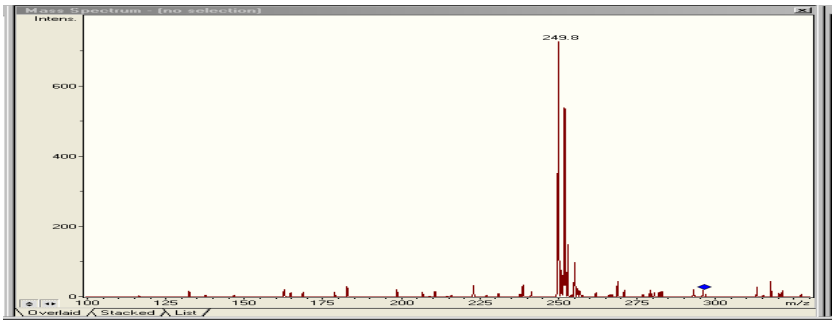


Figure 4. MS/MS spectra of internal standard, DCL

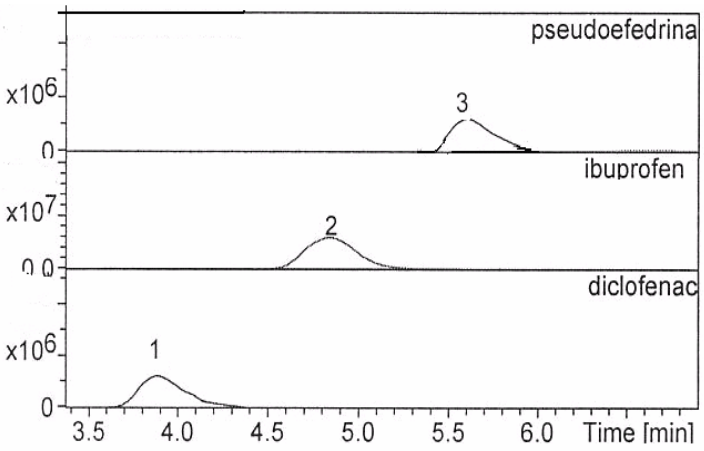


Figure 5. Plasma sample chromatograms of PSE, IBU and DCL at quantification limit (5 ng/ml, respectively 0.05 ng/ml, internal standard, bottom chromatogram)

The calibration curves showed linear response over the range of concentration used in the assay procedure. The calibration curve for IBU was in the concentration range 0.05-60.00 µg/ml, using 8 calibration levels, n = 5 runs with a coefficient of correlation greater than 0.997. The residuals had no tendency of variation with concentration and were between ±14.5% values.

The calibration curve for PSE was in the concentration range 5-300 ng/ml, using 8 calibration levels, n = 5 runs with a coefficient of correlation greater than 0.990. The residuals had no tendency of variation with concentration and were between ±14.7% values.

The inter- and intra-day precision and accuracy results are showed in Table 2 to Table 5 and they are in agreement to international regulations regarding bioanalytical methods validation [12-17].

Precision and accuracy were for IBU from 1.43% to 16.39% for intra-day assay and from 0.34% to 7.98% for inter-day assay. Precision and accuracy were for PSE from 3.12% to 12.8% for intra-day assay and from 0.8% to 13.07 % for inter-day assay. The lower limit of quantification was established at 0.05 μg/ml for IBU and at 5 ng/ml for PSE. Precision and accuracy of IBU at quantification limit were 16.39% and -5.6% for intra-day determinations and 6.56 % and 4% for inter-day determinations, respectively. For PSE, the precision and accuracy at quantification limit were 4.43% and 12.8% for intra-day determinations and 13.07 % and 0.68% for inter-day determinations, respectively.

The recovery was consistent and averaged at 86.81% for IBU and 69.94% for PSE (Table 2 and 4).

Table 2. Intra-day precision, accuracy and recovery (n = 5) for IBU

$c_{nominal}$ μg/ml	Mean c_{found} μg/ml (± S.D.)		CV %	Bias %	Recovery % (± S.D.)	
0.05	0.0472	0.0077	16.39	-5.6	89.21	3.1
30.00	29.567	1.52	5.14	-1.43	85.18	2.5
60.00	61.242	2.05	3.34	2.07	86.03	4.9

Table 3. Inter-day precision, accuracy (n = 5) for IBU

$c_{nominal}$ μg/ml	Mean c_{found} μg/ml (± S.D.)		CV %	Bias %
0.05	0.052	0.34	6.56	4
30.00	29.14	2.16	7.41	-2.86
60.00	60.93	4.86	7.98	1.55

Table 4. Int. Intra -day precision, accuracy and recovery (n = 5) for PSE

$c_{nominal}$ ng/ml	Mean c_{found} ng/ml (± S.D.)		CV %	Bias %	Recovery % (± S.D.)	
5.00	5.64	0.25	4.43	12.8	61.38	4.8
150.00	132.26	4.13	3.12	-11.8	73.89	4.5
300.00	271.42	14.4	5.30	-9.52	74.6	5.6

Table 5. Inter-day precision, accuracy (n = 5) for PSE

$c_{nominal}$ ng/ml	Mean c_{found} ng/ml (± S.D.)		CV %	Bias %
5.00	5.2	0.68	13.07	4.0
150.00	149.96	0.94	0.62	-0.02
300.00	286.88	2.29	0.8	-4.37

Method validation

Method validation [10, 12-17] involves verifying specificity by using six different plasma blanks. The linearity of the peak area against standard concentration was verified between 0.05.00-60.00 µg/ml ibuprofen and between 5-300 ng/ml for PSE by applying least-squares linear regression. The applied calibration model was: y = ax+b, 1/y weight, where y is peak area and x, concentration. Distribution of the residuals (% difference of the back-calculated concentration from the nominal concentration) was investigated. The calibration model was accepted, the residuals were within ±20% at the lower limit of quantification and within ±15% at all other calibration levels and at least 2/3 of the standards met these criteria.

The limit of quantification was established as the lowest calibration standard with an accuracy and precision less than 20%. The intra- and inter-day precision (expressed as coefficient of variation, CV%) and accuracy (relative difference % between found and theoretical concentration, bias %) of the assay procedure were determined by the analysis in the same day of three samples at each of three levels of concentration in the considered concentration range and one sample of each in three different days, respectively. The recoveries at each of previous levels of concentration were measured by comparing the response of the treated plasma standards with the response of standards in water with the same concentration in ibuprofen as the final extract from plasma standards.

The stability of the stock solutions of IBU, PSE and DCL was checked and found acceptable within 1.36% to 5.39% for 4 weeks of storage in the refrigerator at 4 to 8 ^{o}C. The freeze and thaw stability of the analytes IBU and PSE in plasma was checked during three cycles of freeze at -60 ^{o}C and thaw at room temperature.The results are presented in table 6 and 7.

Post-Preparative Stability is a procedure designed to measure the analyte stability in samples over the anticipated run time for the batch size, including the resident time in the autosampler by determining concentrations on the basis of original calibration standards. Post-preparative stability was checked for IBU and PSE, for 24 h of keeping the samples at 4^{o}C in the HPLC thermostated autosampler. The results were within 5.15% to 11.6% for IBU respectively within 8.5 to 10.9% for PSE.

Short-term room temperature stability was checked by preparing at least three spiked plasma aliquots of concentration 0.15 µg/ml IBU, 10 ng/ml PSE and 60 µg/ml IBU, 300 ng/ml PSE. These samples were stored at room temperature for 3 hours, then extracted and analysed against calibration together with freshly prepared samples of the same concentration and number. Mean concentration was calculated for the stored and the reference samples and compared. The results were between 2.34 and 9.32 for IBU and between 13.67 and 13.91 for PSE.

Table 6. Freeze-thaw stability (n = 3) for IBU

$c_{nominal}$ µg/ml	Freshly prepared	After the 3rd cycle	Bias %
0.15	0.148	0.154	3.64
60	54.05	51.3	5.08

Table 7. Freeze-thaw stability (n = 3) for PSE

$c_{nominal}$ ng/ml	Freshly prepared	After the 3rd cycle	Bias %
10	9.46	10.43	10.2
300	307.2	289.03	5.91

CONCLUSIONS

The proposed method is simple and sensitive and provides accuracy and precision in the simultaneously determination of IBU and PSE. The lower limit of quantification was established at 0.05 µg/ml IBU and 5ng/ml PSE, respectively, with accuracy and precision less than 16.4%. The MS/MS detection and the simple sample preparation method allowed a specific and efficient analysis of a large number of plasma samples from the clinical study. The developed method is suitable for quantification of IBU and PSE in human plasma for a bioequivalence study after administration of a solid dosage form combination of both to healthy subjects.

EXPERIMENTAL SECTION

Reagents

IBU, PSE and DCL were European Pharmacopoeia standard. Diethyl ether, hexane, methanol, orto phoshoric acid, sodium hydroxide and ammonium acetate were Merck products (Merck KgaA, Darmstadt, Germany). Distilled, deionised water was produced by a Simplicity 185 Millipore (Millipore SA,

Molsheim, France) water system. The human blank plasma was supplied by the Local Bleeding Centre Cluj-Napoca, Romania.

Standard solutions

Two stock solutions of IBU and PSE, respectively, with concentration of 10 µg/ml and respectively 100 µg/ml were prepared by dissolving appropriate quantities of reference substances in methanol. The stock solution of internal standard DCL was prepared by dissolving the suitable quantity of substance in methanol to obtain a solution of 103 µg/ml. Ten plasma standards with the concentrations ranged between 0.05 and 60 µg/ml for IBU and respectively 5 and 300 ng/ml PSE were prepared. Accuracy and precision of the method was verified using plasma standards with concentrations of 0.05, 30 and 60 µg/ml IBU and 5, 150, 300 ng/ml PSE, respectively. The concentration of internal standard DCL in plasma was 10 µg/ml.

Chromatographic and mass spectrometry systems and conditions

The HPLC system was an 1100 series model (Agilent Technologies, Darmstadt, Germany) consisted of a binary pump, an in-line degasser, an autosampler, a column thermostat, and an Ion Trap VL mass spectrometer detector (Brucker Daltonics GmbH, Bremen, Germany). Chromatograms were processed using QuantAnalysis software version 5.1 (Brucker Daltonics). The detection of IBU was in MRM mode, using electrospray negative ionization, the ion transition monitored being m/z 205> m/z (159+161). The detection of PSE was in MRM mode using electrospray positive ionization, the transition monitored being m/z 166>148. The detection of internal standard was in MRM mode, using electrospray negative ionization by monitoring the transition m/z 293> m/z 250 m/z.

Mobile phase

The mobile phase consisted of a mixture methanol: ammonium acetate 10 mM in water =60:40, v/v each component being degassed, before elution, for 10 min in an Elma Transsonic 700/H (Singen, Germany) ultrasonic bath. The pump delivered the mobile phase at 0.3 ml/min. The chromatographic separation was performed in less than 8 minutes, at 20C on a Zorbax RX C18 3.0 x 100mm, 3µ column (Agilent Technologies), protected by an in-line filter.

Sample preparation

For chromatographic analysis standard and test plasma samples were prepared as follows. After adding 1.4N phosphoric acid (100 µl) into a previously vortexed (5 s at 1500 rpm) mixture of plasma (1000 µl) and internal

standard (DCL, 100 µl), the resulted solution was vortex-mixed for 5 s at 1500 rpm. The solution was extracted with a mixture of diethyl ether: hexane (5 ml, 80:20, v/v) by shaking for 25 min. at 1000 rpm. The resulted mixture was centrifuged (5000 rpm) and the organic layer (4 ml) was isolated. The extracted water solution was treated with 2M sodium hydroxide (275 µl), vortex-mixed (5 s at 1500 rpm) and was subjected for extraction as described above. The combined organic layer (2×4 ml) was evaporated to dryness at 50 °C under a stream of nitrogen. The residue was suspended in the mobile phase (200 µl), vortexed (1 min. at 2400 rpm), centrifuged and injected into HPLC.

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