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Simultaneous Determination of Hyoscine, Ketoprofen and Ibuprofen in Pharmaceutical Formulations by HPLC - DAD

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ABSTRACT

The objective of the work was to establish a new, rapid and sensitive HPLC–DAD method for simultaneous determination of three most commonly prescribed drugs; hyoscine, ketoprofen, and ibuprofen. The HPLC separation of the analytes was performed on a hypersil - Gold C18 (150 mm × 4.6 mm, 8 µm) column, using gradient elution of the mobile phase composed of 0.01 M potassium phosphate dibasic containing 2 g/L heptane sulphonic acid sodium salt maintained at pH 3.5 (Pump A) and acetonitrile 80% v/v (pump B) with a flow rate of 2 mL/min. The multiple wavelength detector was set at 210 nm for measurement of all compounds. Quantification was based on measuring the peak areas. The three compounds were resolved with retention times 6.42 ± 0.009, 10.63 ± 0.006 and 16.43 ± 0.008 min for hyoscine, ketoprofen and ibuprofen, respectively.The calibration curves were linear in the range of 0.64 – 96, 0.64 – 400 and 1.28 – 640 µg/mL for hyoscine, ketoprofen and ibuprofen, respectively, all of them with coefficients of determination above 0.9995. The methodology recoveries were higher than 95.0%. The limits of detection (LODs) were 0.11, 0.17 and 0.17 µg/mL for hyoscine, ketoprofen, and ibuprofen, respectively. The intra- and inter-day coefficients of variation were less than 2%. The method is accurate, sensitive and simple for quality control as well as for stability indicating purposes.

INTRODUCTION

The work proposed in this study involves the analysis of three drugs; hyoscine butyl bromide and two representative examples of non steroidal anti inflammatory drugs among the profens; ketoprofen and ibuprofen. The high importance of these drugs resulted from their widespread use and the possibility of being administered concurrently. This prompted us to review the most important recent methods for their analysis in pure form, in different pharmaceutical dosage forms and in biological fluids reported so far in the literature. It also encourages us to develop a reliable method for their determination. Because of the large number of references that appeared as individual methods or as part of clinical and pharmacological studies, it is possible to make reference only to the most important papers. Hyoscine N- butylbromide (HSC) (-)-(1S,3s,5R,6R,7S,8r)-6,7-epoxy-8-butyl-3-[(S)-tropoyloxy]tropanium bromide (fig. 1) is a tertiary amine antimuscarinic agent with central and peripheral actions.

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HSC has been used as an antispasmodic drug to relieve the pain of smooth muscle spasm associated with the gastrointestinal tract. It may also be useful in diagnostic procedures of the gastrointestinal tract. HSC is also an effective agent in the prevention of motion sickness and is used in the prevention of postoperative nausea and vomiting (Sweetman, 2009). HSC is official in BP 2012 (2012) where a non aqueous titration method is described for its analysis. Many analytical reports were found in the literature for the determination of HSC including spectrophotometric methods (Gouda, 2010; Gouda et al., 2008; Issa et al., 2005), HPLC (Nakagawa et al., 2000; Parissi-Poulou and Panderi, 1999; Wang and Zhu, 2000), capillary electrophoresis (Chang et al., 2000; Cherkaoui et al., 1999) and electrochemical methods (Abramovic et al., 2005; El-Saharty et al., 2007b) among other methods. HSC is formulated and analyzed in mixture with other pharmaceuticals such as, oxazepam (Toral et al., 2005), medazepam (Karali et al., 1998) and with the non-steroidal anti-inflammatory drug ketoproen where two reports were found in the literature for the determination of this mixture either in pharmaceutical preparations using derivative, derivative ratio, bivariate calibration spectrophotometric methods and reversed phase LC coupled with UV detection

(El-Saharty et al., 2007a) as well as in plasma and in formulations using electrochemical methods (El-Saharty et al., 2007b). Ketoprofen (KTP) (RS)-2-(3-benzoylphenyl)propionic acid and Ibuprofen (IBP) 2-(4-isobutylphenyl)propionic acid, both are propionic acid derivatives (fig. 1). They are non-steroidal anti-inflammatory drugs (NSAID); they are given as racemic mixtures. They are used in the management of mild to moderate pain in conditions such as dysmenorrhoea, headache including migraine, postoperative pain, dental pain, musculoskeletal and joint disorders such as osteoarthritis, and rheumatoid arthritis. They are also used to reduce fever (Sweetman, 2009). The investigated drugs are official in BP 2012 (2012) which describes titrimetric methods for their analysis. They are also official in USP 34 (United States Pharmacopeial Convention. Committee of Revision., 2011), where KTP is analyzed through a titrimetric procedure; whereas an HPLC procedure has been described for the analysis of IBP. The analytical profiles of IBP and KTP have been reviewed (Higgins et al., 2001; Liversidge, 1981). A full review of spectrophotometric and spectrofluorimetric methods for the determination of NSAID including profens; specifically KTP and IBP has been published by A.A. Gouda et al (Gouda et al., 2013), This review covers the time period from 1985 to 2010 during which 145 spectrophotometric methods including UV , derivative, visible methods based on formation of metal complexation, redox reactions, ion pair formation, charge-transfer complexation and miscellaneous; flow injection spectrophotometry as well as spectrofluorometric methods were reviewed. KTP has been determined through chemiluminescence (Zhuang and Song, 2007) in capsules and human urine samples. Two flow injection spectrometric methods have been reported for the analysis of KTP (Aboul-Enein et al., 2003; Ozlu et al., 2005). KTP enantiomers have been analyzed and resolved by HPLC (Oda et al., 1992), the simultaneous determination of KTP, in presence of two preservatives methylparaben and propylparaben and two of its degradation products has been achieved using HPLC, UV detection (Dvorak et al., 2004). A highly sensitive UPLC–MS/MS method for the quantitative analysis of KTP in dialyzes from topical preparations has been published (Tettey-Amlalo and Kanfer, 2009). KTP has been determined simultaneously with other pharmaceuticals, from these we can mention GC- MS/MS for the analysis of KTP and other NSAID including IBP, and/or naproxen in waste water and environmental water samples, after derivatization (Hashim and Khan, 2011; Yu et al., 2012), GC-MS/MS in urine and blood samples (Azzouz and Ballesteros, 2012), the use of capillary electrochromatography coupled with UV or mass spectrometry in water samples (Hsu et al., 2011), GC-tandem mass spectrometry in bovine milk (Dowling et al., 2008), HPLC for the simultaneous determination of KTP and mefenamic acid in tablets (Hung and Hwang, 2008), with IBP and other NSAID (Jedziniak et al., 2012) (Patrolecco et al., 2013). A simple new chemiluminescent method for the determination of IBP and KTP is described using the Fenton system in the presence of europium(iii) ions (Kaczmarek and Lis, 2012). Studies for the determination of IBP include: spectrophotometry using compensation method, derivative methods and two wavelengths methods (Jain et al., 2011; Wahbi et al., 2005) , complexation with phenolphthalein-β-cyclodextrin (Afkhami et al., 2007), spectrofluorimetrically (Damiani et al., 2001), G.C. (Guo et al., 2009) and HPLC (Zheng and Chen, 2007). It has also been determined in biological fluids using HPLC-UV (Farrar et al., 2002), micellar LC (Shi et al., 2010) and LC-MS (Li et al., 2008).
IBP has been determined simultaneously in mixtures with paracetamol; using HPLC (Zhang et al., 2005), chemometric-assisted spectrophotometric methods (Hassan, 2008), with some NSAIDs including KTP using capillary electrophoresis (Chen and Wu, 2005), with pseudoephedrine HCl using derivative ratio spectrophotometry (Palabiyik et al., 2004) or HPLC (Langlois et al., 2009; Zhao et al., 2003) ,with paracetamol and methocarbamol using RP-HPLC (Vasudevan et al., 2000), with flurbiprofen using capillary zone electrophoresis(Hamoudova and Pospisilova, 2006), with sodium phenobarbital using HPLC (Chang and Zhang, 2009), with diphenhydramine citrate using LC (Rao et al., 2009), in presence of its degradation products using HPLC (Chayeh et al., 2007), with famotidine in tablets using second order derivative spectrophotometry (Shah et al.), with famotidine and paracetamol in tablets using stability indicating HPTLC method (Dubey et al., 2012).

EXPERIMENTAL

Instrumentation

The HPLC-DAD system consisted of Shimadzu Prominence Liquid Chromatograph (Shimadzu Corporation, Tokyo, Japan), quaternary pump (LC20 AD), Prominence degasser (DGU 20 As) and Prominence diode array and multiple wavelength detector (SPD-M 20 A) connected through a Prominence communication bus Module (CBM 20 A).

A prominence auto sampler (SIL 20 AC) with variable sample injection volume was used. The column used was Hypersil Gold C18 (4.6 × 150 mm, 8 µm particle size), the column temperature was maintained at 30 ºC using a prominence column Oven (CTO 20 AC).

Materials and Reagents

Hyoscine butylbromide (HSC), Ketoprofen (KTP) and Ibuprofen (IBP) were all kindly donated by Amriya Pharmaceuticals Co., Alexandria, Egypt. HPLC-grade acetonitrile (FUV) (Lab Scan Analytical Sciences, Poland), Heptane sulphonic acid sodium salt (Fisher Scientific, UK). Puriss analytical grade of potassium phosphate dibasic (K_2HPO_4), hydrochloric acid, phosphoric acid, sodium hydroxide pellets, hydrogen peroxide 30% and high purity bidistilled water were used. Diluting solvent was prepared as mixture of 50% acetonitrile and 50% 0.01 M K_2HPO_4 adjusted to pH 3.5 using phosphoric acid. Pharmaceutical preparations assayed through the study are Spasmofen® ampoules labeled to contain 20 mg HSC and 100 mg KTP, manufactured by Amriya Pharmaceuticals Co., Alexandria, Egypt. Brufen® tablets labeled to contain 400 mg IBP, manufactured by Kahira Pharm. &

Chem. Ind. Co. Egypt under license of Abbott Laboratories. Biprofenid® Tablets labeled to contain 150 mg KTP, manufactured by Sanofi-aventis, Egypt under license of Sanofi-aventis, France. Buscopan® tablets labeled to contain 10 mg HSC, and manufactured by Chemical Industries Development (CID), Egypt under license of Boehringer Ingelheim International GmbH, Germany.

General procedure

Chromatographic conditions

A mobile phase system consisting of 0.01 M K_2HPO_4 containing 2 g/L Heptane Sulphonic acid sodium salt (HSASS) maintained at pH 3.5 (Pump A) and acetonitrile, 80 % v/v (Pump B) was used. The separation was achieved with the linear gradient program stated in Table 1. The flow rate was 2.0 mL/min. The injection volume was 50 µL. The eluant was monitored by the diode array detector from 190 to 400 nm, and chromatograms were extracted at 210 nm. All determinations were performed at 30 ºC.

Table. 1: Gradient Study Used in the Study.

Time (min)	Pump A %	Pump B %	Comment
0	75	25	Start up
20.00	35	65	Linear Gradient
20.01	75	25	Restabilization
22	75	25	Stop

Standard solutions

HSC (128 µg/mL), KTP (1000 µg/mL) and IBP (2000 µg/mL) stock solutions were prepared in diluting solvent mixture. The working solutions were prepared by dilution of aliquots of the stock solutions with the diluting solvent to reach the concentration ranges 0.64 - 96, 0.64 - 400 and 1.28 - 640 µg/mL for HSC, KTP and IBP, respectively. Triplicate injections were made for each concentration and chromatographed under the described LC conditions. The peak areas were plotted against the corresponding concentrations to construct the calibration graphs.

Assay of pharmaceutical products

Ten tablets of each preparation were weighed and finely powdered. For each preparation, 50 mL diluting solvent was added to a quantity of the powdered tablets equivalent to 12.8 mg HSC, 100 mg KTP and 200 mg IBP; the solutions were stirred and sonicated for 15 min, and then filtered into 100- mL volumetric flasks. The residues were washed with 2 × 10 mL diluting solvent mixture, and the washings were added to the filtrate and diluted to the final volume with the same diluting solvent mixture. Aliquots of each tablet solution were diluted with the solvent mixture to reach final concentrations within the specified ranges and then treated as under "General Procedure".

For spasmofen ampoules® 160 µL of Spasmofen ampoules were accurately transferred into a 25- mL volumetric flask using calibrated micropipettes. The volume was completed with diluting solvent, and chromatographed as described under "general procedure". Recovered concentrations were calculated from the corresponding calibration graphs.

Forced degradation and stability-indicating study

Forced degradation studies were carried out on HSC, KTP and IBP standards according to the following conditions:

(a) *Acidic and basic conditions*

HSC, KTP and IBP solutions were treated with 5 mL of 1 M HCl or 1 M NaOH. The solutions were placed in a water bath at 80 °C for 30 min. After the specified time, all solutions were neutralized by adjusting the pH to 7.0 and then diluted to volume with diluting solvent.

(b) *Neutral hydrolysis*

HSC, KTP and IBP solutions were placed in a water bath at 80 °C for 30 min. After the specified time, all solutions were diluted to volume with diluting solvent.

(c) *Oxidation with H_2O_2*

HSC, KTP and IBP solutions were treated with 5 mL of hydrogen peroxide 30%. The solutions were placed in a water bath at 80 °C for 30 min, and then the solutions were diluted to volume with diluting solvent.

(d) *Photolytic degradation*

HSC, KTP and IBP solutions were subjected to UV irradiation at 254 nm for 1 hr. and then the solutions were diluted to volume with diluting solvent. All solutions were then filtered with a 0.45 µm filtration disk prior to injection to the column.

RESULTS AND DISCUSSION

A stability-indicating HPLC-DAD method was developed to provide rapid and reliable quality control analysis of three drugs that are most often used in human and veterinary medicine since they are available without prescription (Warden, 2010) for treatment of minor conditions such as fever, headache and dysmenorrhea. The work proposed in this paper was directed towards the simultaneous determination of these mentioned drugs in their single and/or combined formulations. Forced-degradation studies should be considered during development of chromatographic procedures particularly when degraded products are unknown or not available (ICH, 1993). Hence, forced-degradation experiments were carried out on HSC, KTP and IBP in order to produce the possible relevant degradants and test their chromatographic behavior using the developed HPLC method. Hydrolytic (using neutral, strong acidic and basic media) and oxidative and photolytic degradation studies were conducted. The fact that up till now the simultaneous determination of the three drugs has not been reported in the literature has encouraged us to develop an HPLC- DAD stability indicating assay where the decomposition products were resolved from the intact drugs.

Optimization of chromatographic conditions

The mobile phase system optimized in this study consisted of (A) 0.01 M K_2HPO_4 containing 2 g/L HSASS maintained at pH 3.5 and (B) acetonitrile 80% v/v with a linear

gradient program. Several trials in the isocratic mode as well as gradient programs were tried and further optimized, concerning the ratio of acetonitrile to the aqueous phase in the system as well as the time of gradient program, so as to fulfill the requirement of resolving the three drugs from each other and from their stress degradation products. Acetonitrile in high concentration was an important factor that led to the elution of IBP in a reasonable retention time with acceptable peak asymmetry. On the other hand, using the mobile phase with a high proportion of acetonitrile in the gradient mode resulted in a complication for the relatively quickly eluting HSC peak. To overcome this conflict and to ensure complete resolution of the active ingredients from other extra forced degradation peaks, the best compromise among adequate resolution, reasonable retention times, and tolerable peak asymmetry was achieved using a gradient system starting with 25% (v/v) pump B ramped up linearly 2% acetonitrile per min to 65% in 20.00 min, at 20.01 min acetonitrile was returned to the initial value and then stabilized at this percentage afterwards till the end of the program at 22 min. The effect of flow rate was studied, and 2.0 mL/min was found optimum regarding run time, peak asymmetry, and column pressure and was kept constant throughout the gradient program. The analytes were measured using the DAD at 210 nm ensuring method specificity by comparison of recorded spectra during peak elution. Quantification was achieved based on peak area measurement. The previously described chromatographic conditions were established with a view to develop a stability indicating assay method where HSC, KTP and IBP were resolved with symmetrical peaks; fig. 2 shows a typical chromatogram for the separation of the three drugs. Moreover, they gave good separation between each of the three drugs and their stress degradation products. System suitability parameters were optimum and are listed in Table 2.

Table. 2: System suitability parameters for the HPLC – DAD determination of HSC - KTP - IBP mixture.

Parameter	HSC	KTP	IBP
t_R ± SD (min)	6.42 ± 0.009	10.63 ± 0.006	16.43 ± 0.008
Capacity factor (k′)	6.23	10.97	17.51
Theoretical plates (N)	2421.44	17933.19	33114.10
Selectivity (α)		1.76	1.60
Resolution (R_s)		10.05	17.08

Stability indicating aspects

Forced-degradation experiments were carried out on each of the three drugs in this combination in order to produce the possible relevant degradants and test their chromatographic behavior using the developed method. Hydrolytic (using neutral, strong acidic and basic media), oxidative degradation and photolytic degradation studies were conducted under specified conditions of time and temperature. A Summary of degradation studies of HSC-KTP-IBP using the proposed HPLC method has been presented in Table 3. All the three drugs show slight degradation under different stress conditions applied throughout the study. Minor peaks appeared at different retention. The gradient program time was extended till 22 min to ensure the elution of all related degradation peaks. Representative HPLC chromatograms for the three drugs after oxidative degradation were shown (fig. 3). The analyzed drugs appeared at their specific retention times with areas almost identical to that of a standard of the same concentration. In all these experiments, resolution was calculated between any of the three analytes and the nearest degradation products peaks. Resolution was found not less than 2; this implies an adequate baseline separation between the main drugs and any of the degradation products. It is noteworthy to mention that peak purity test results obtained from the diode-array detector (DAD) confirm that HSC, KTP and IBP peaks are homogenous and pure in all the analyzed samples subjected to forced degradation conditions.

Table. 3: Summary of degradation studies of HSC-KTP-IBP using the proposed HPLC method.

Analyte	Degradation conditions	Degradation (%)
HSC	Acidic hydrolysis 80°C for 30 min	2.14 %
KTP		0.98 %
IBP		0.45 %
HSC	Basic hydrolysis 80°C for 30 min	0.11 %
KTP		0.33 %
IBP		0.27 %
HSC	Neutral hydrolysis 80°C for 30 min	4.46 %
KTP		0.18 %
IBP		0.40 %
HSC	Photo-degradation UV at 254 nm for 1 h	0.84 %
KTP		0.15 %
IBP		0.42 %
HSC	Oxidative degradation 80° C for 30 min	1.25 %
KTP		0.80 %
IBP		1.52 %

Validation of the proposed method

Linearity and concentration ranges

The linearity of the proposed HPLC procedure was evaluated by analyzing a series of different concentrations for each of the three analytes. The linear regression equations were generated by least squares treatment of the calibration data. Under the optimized conditions described above, the measured peak areas were found to be proportional to concentrations of the analytes. Table 4 presents the performance data and statistical parameters including linear regression equations, concentration ranges, correlation coefficients, standard deviations of the intercept (S_a), slope (S_b) and standard deviations of residuals ($S_{y/x}$). Regression analysis shows good linearity as indicated from the correlation coefficient values (>0.9995). In addition, deviation around the slope can be further evaluated by calculation of the RSD% of the slope (S_b%) which were found to be less than 1.2%. The analysis of variance test for the regression lines reveals that, for equal degrees of freedom, an increase in the variance ratio (F values) means an increase in the mean of squares due to regression and a decrease in the mean of squares due to residuals. The greater the mean of squares due to regression, the steeper is the regression line. The smaller the mean of squares due to residuals, the less is the scatter of experimental points around the regression line. Consequently, regression lines with high F values (low significance F) are much better than those with lower ones. Good regression lines show high values for both r and F statistical parameters (Armitage et al., 2008).

Detection and quantification limits

According to the pharmacopoeial recommendations, the limit of detection is defined as the concentration that has a signal-to-noise ratio of 3:1, while for limit of quantification, the ratio considered is 10:1. The LOD and LOQ values for the three drugs were calculated and presented in Table 4.

Table. 4: Analytical parameters for the determination of HSC-KTP-IBP mixture using the proposed HPLC-DAD method.

Parameter	HSC	KTP	IBP
Wavelength (nm)	210	210	210
Concentration range(μg/mL)	0.64 – 96	0.64 - 400	1.28 – 640
Intercept (a)	-4403.61	-385720.50	-410035.31
S_a[a]	7692.11	225523.50	210183.20
Slope (b)	21808.74	124042.94	60221.21
S_b[b]	158.67	1248.76	710.01
RSD% of the slope (S_b%)	0.73	1.01	1.18
Correlation coefficient (r)	0.99979	0.99954	0.99951
$S_{y/x}$[c]	16450.42	550038.03	462151.70
F[d]	18892.53	9867.02	7193.91
Significance F	8.78×10^{-15}	5.39×10^{-15}	8.34×10^{-12}
LOD[e] (μg/mL)	0.11	0.17	0.17
LOQ[f] (μg/mL)	0.40	0.56	0.56

[a] Standard deviation of the intercept
[b] Standard deviation of the slope
[c] Standard deviation of residuals
[d] Variance ratio, equals the mean of squares due to regression divided by the mean of squares about regression (due to residuals)
[e] Limit of detection
[f] Limit of quantification

Precision and accuracy

The within-day (intra-day) precision and accuracy for the proposed method were studied at three concentration levels for each compound using three replicate determinations for each concentration within one day. Similarly, the between-day (inter-day) precision and accuracy were tested by analyzing the same three concentrations for each compound using three replicate determinations repeated on three days. Recoveries were calculated using the corresponding regression equations and they were satisfactory. The percentage relative standard deviation (RSD %) and percentage relative error (E_r %) did not exceed 2.0 % proving the high repeatability and accuracy of the developed method for the estimation of the analytes in their bulk form (Tables 5 and 6).

Selectivity and specificity

Method selectivity was examined by preparing several laboratory-prepared mixtures of the three compounds at various concentrations within the linearity ranges mentioned in Table 4. The laboratory-prepared mixtures were analyzed according to the previously described procedure. The analysis results including percentage relative standard deviation (RSD %) and the percentage relative error (E_r %) values shown in Table 7 were satisfactory thus validating the selectivity, precision and accuracy of the developed method and demonstrating its capability to resolve and quantify the analytes in different ratios. Specificity is defined as the ability to access unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products and matrix components (United States Pharmacopeial Convention. Committee of Revision., 2011), and this is well demonstrated in details through the analysis of pharmaceutical dosage forms and forced degradation studies. No extra peaks were observed from any of the inactive ingredients in the dosage forms. Also, the DAD enables peak purity verification, where no signs of co-elution from any of the inactive components were detected. Selectivity was demonstrated by separation of the three analytes from their hydrolytic and oxidative degradation products.

Robustness

Robustness was examined by evaluating the influence of small variations in different conditions such as ratio of organic modifier (± 5% acetonitrile), flow rate (± 0.5 mL/min), column temperature (± 5 ºC) and pH value (± 0.5 pH unit). These variations did not have any significant effect on the measured responses or the chromatographic resolution. RSD% for the measured peak areas using these variations did not exceed 1%, results are presented in Table 8.

Stability of solutions

The stability of standard working solutions as well as sample solutions in the diluting solvent mixture was examined and no chromatographic changes were observed within 24 hours at room temperature. The solutions remained unchanged and no significant degradation was observed during this period.

Analysis of pharmaceutical dosage forms

The optimized HPLC-DAD procedure was applied for the assay of these drugs in the available single and combined pharmaceutical formulations. The active ingredients were extracted with the same diluting solvent mixture used for the preparation of the standard stock solutions to reach concentration levels within the specified ranges. The active ingredients eluted at their specific retention times. No interfering peaks were observed from any of the inactive ingredients or the colored coat of the analyzed tablets. The diode-array detection enables peak purity verification where no signs of co-elution from any of the inactive adjuvants were detected (fig. 4).

The assay results revealed satisfactory accuracy and precision as indicated from % recovery, SD and RSD% values (Table 9). Furthermore, different reference HPLC methods were applied for the estimation of the analytes in their single or combined formulations. Recovery data obtained from the developed HPLC method were statistically compared with those of the reference methods using the Student's *t*- and the variance ratio *F*-tests. In both tests, the calculated values did not exceed the theoretical ones at the 95% confidence level which indicated that there were no significant differences between the recoveries obtained from the developed method and those of the reference methods (Table 9). It is evident from these results that the proposed method is applicable to the assay of these drugs combinations with satisfactory level of selectivity, accuracy and precision.

Table . 5: Accuracy results for the determination of HSC, KTP and IBP in bulk form using the proposed HPLC-DAD method.

Analyte	Nominal value (µg/mL)	Found ± SD[a] (µg/mL)	RSD(%)[b]	E_r(%)[c]
HSC	16	16.11 ± 0.08	0.52	0.01
	32	32.15 ± 0.05	0.15	0.02
	48	48.47 ± 0.11	0.23	0.05
	64	64.75 ± 0.002	0.003	0.08
	80	81.25 ± 0.16	0.19	0.13
KTP	80	81.04 ± 0.01	0.01	0.10
	160	159.93 ± 0.02	0.01	-0.01
	240	235.68 ± 0.27	0.12	-0.43
	320	320.52 ± 0.09	0.03	0.05
	400	404.39 ± 1.16	0.29	0.44
IBP	160	161.1 ± 0.03	0.02	0.11
	320	322.16 ± 0.28	0.09	0.22
	480	472.77 ± 0.11	0.02	-0.72
	640	651.85 ± 0.26	0.04	1.18

[a] Mean ± standard deviation for five determinations.
[b] % Relative standard deviation.
[c] % Relative error.

Table. 6: Precision Results For The Determination Of Hsc, Ktp And Ibp In Bulk Form Using The Proposed Hplc-Dad Method.

Analyte	Nominal value (µg/mL)	Within-day			Between-day		
		Found ± SD[a] (µg/mL)	RSD(%)[b]	E_r(%)[c]	Found ± SD[a] (µg/mL)	RSD(%)[b]	Er(%)[c]
HSC	64	63.94 ± 0.93	1.45	- 0.09	64.12 ± 0.94	1.47	0.20
KTP	320	319.44 ± 4.79	1.50	- 0.18	319.70 ± 4.90	1.53	- 0.09
IBP	640	642.57 ± 11.91	1.85	0.40	642.43 ± 12.09	1.88	0.38

[a] Mean ± standard deviation for five determinations.
[b] % Relative standard deviation.
[c] % Relative error.

Table. 7: Determination of HSC-KTP-IBP laboratory-prepared mixtures using the proposed HPLC-DAD method.

Nominal value (µg/mL)			Found ± SD[a] (µg/mL)			RSD(%)[b]			E_r(%)[c]		
HSC	KTP	IBP	HSC	KTP	IBP	HSC	KTP	IBP	HSC	KTP	IBP
16	320	640	16.38	315.02	641.43	1.31	0.88	0.80	2.35	-1.56	0.22
32	320	640	30.63	318.49	643.63	0.70	0.87	0.79	- 4.28	-0.47	0.57
48	320	640	47.43	314.77	642.21	0.45	0.88	0.79	-1.19	-1.64	0.35
64	320	640	63.94	321.17	642.44	0.34	0.86	0.79	-0.10	0.36	0.38
80	320	640	82.83	319.58	661.60	0.26	0.87	0.77	3.54	-0.13	3.37
96	320	640	95.34	320.61	642.04	0.23	0.87	0.79	-0.69	0.19	0.32
64	80	640	63.69	77.49	650.00	0.34	3.58	0.78	-0.49	-3.14	1.56
64	160	640	63.75	154.39	641.90	0.34	1.80	0.79	-0.39	-3.51	0.30
64	240	640	64.18	233.89	647.00	0.34	1.19	0.79	0.28	-2.55	1.09
64	320	640	63.94	321.39	642.44	0.34	0.86	0.79	-0.10	0.44	0.38
64	400	640	63.78	413.85	635.12	0.34	0.67	0.80	-0.34	3.46	-0.76

[a] Mean ± standard deviation for five determinations.
[b] % Relative standard deviation.
[c] % Relative error.

Table. 8: Data for robustness study.

Condition	HSC		KTP		IBP	
	Mean area ± SD[a]	RSD(%)[b]	Mean area ± SD[a]	RSD(%)[b]	Mean area ± SD[a]	RSD(%)[b]
STD	1325266.60 ± 669.76	0.05	39112117.80 ± 156937.30	0.40	37091244.40 ± 272477.71	0.73
Low Organic	1330046.20 ± 390. 53	0.03	37877899 ± 218470.17	0.58	35893749.60 ± 15520.67	0.04
High Organic	1325410.20 ± 343.51	0.03	39183881.60 ± 45585.83	0.12	36729195.40 ± 100914.69	0.27
Low Flow	1778414 ± 3539.72	0.20	55361962.20 ± 198040.04	0.36	54057770.40 ± 178463.40	0.33
High Flow	1067002.60 ± 1748.50	0.16	28784006 ± 23601.32	0.08	28309507.60 ± 7828.54	0.03
Low pH	1332885.20 ± 1218.23	0.09	37775358.80 ± 50544.17	0.13	35792262.40 ± 18261.78	0.05
High pH	1328874.60 ± 459.23	0.03	38055211.40 ± 32347.97	0.09	35927590.20 ± 17040.59	0.05
Low Temp.	1325705 ± 487.61	0.04	38137413.80 ± 80738.44	0.21	36053649.20 ± 88489.22	0.25
High Temp.	1327313 ± 1988.75	0.15	38342573.60 ± 39663.72	0.10	36286737.40 ± 44943.62	0.12

Table . 9: Application of the proposed HPLC-DAD method to the analysis of HSC-KTP-IBP mixture in pharmaceutical preparations.

Preparation	Proposed method		Reference method[c]	
	%Recovery ± SD[a]	RSD%[b]	%Recovery ± SD[a]	RSD%[b]
Spasmofen® ampoules HSC 20 mg/ 2mL	101.71 ± 1.74; t = 1.40 , *F*= 3.74	1.71	102.83 ± 0.90	0.87
KTP 100 mg/ 2 mL	100.24 ± 0.76; t = 0.09 , *F*= 4.51	0.75	100.28 ± 0.36	0.36
Buscopan® tablets 10 mg HSC/ tablet	99.86 ± 0.60; t = 0.60 , *F*= 1.92	0.60	99.61 ± 0.83	0.83
Bi- profenid® tablets 150 mg KTP/ tablet	98.93 ± 1.07; t = 0.41 , *F*= 3.16	1.07	98.57 ± 1.90	1.92
Brufen® tablets 400 mg IBP/ tablet	100.93 ± 1.16; t = 1.81 , *F*= 1.71	1.15	99.85 ± 0.89	0.89

[a] Mean ± standard deviation for five determinations.
[b] % Relative standard deviation.
[c] HPLC BP methods.
Theoretical values for t and F at P = 0.05 are 2.26 and 5.05, respectively.

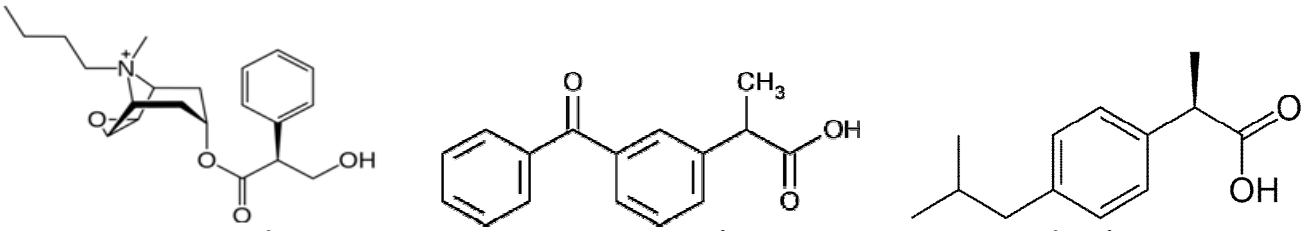

Fig. 1: Chemical structures of hyoscine (HSC), ketoprofen (KTP) and ibuprofen (IBP).

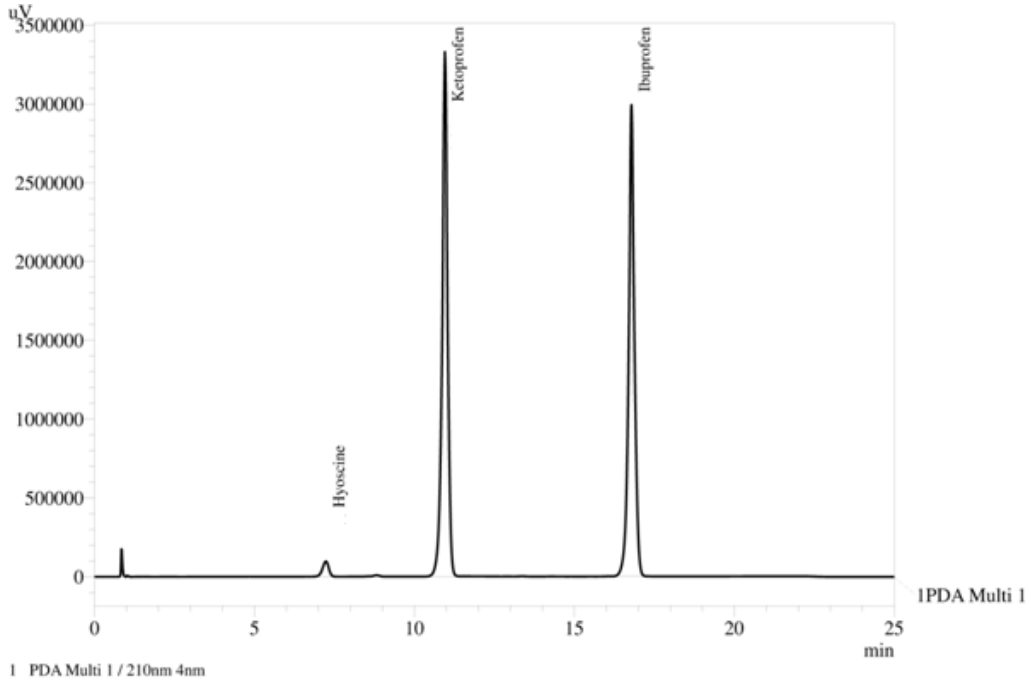

Fig. 2: HPLC chromatogram of 50 µL injection of a mixture containing 64 µg/mL HSC, 320 µg/mL KTP and 640 µg/mL IBP at 210 nm.

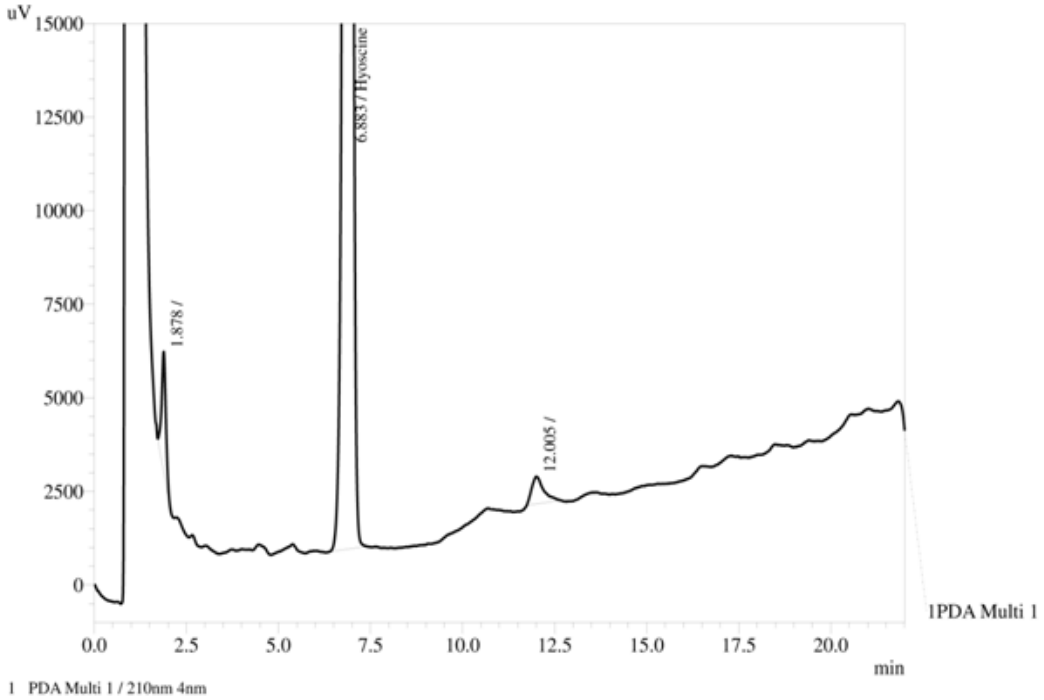

Fig. 3. (A)

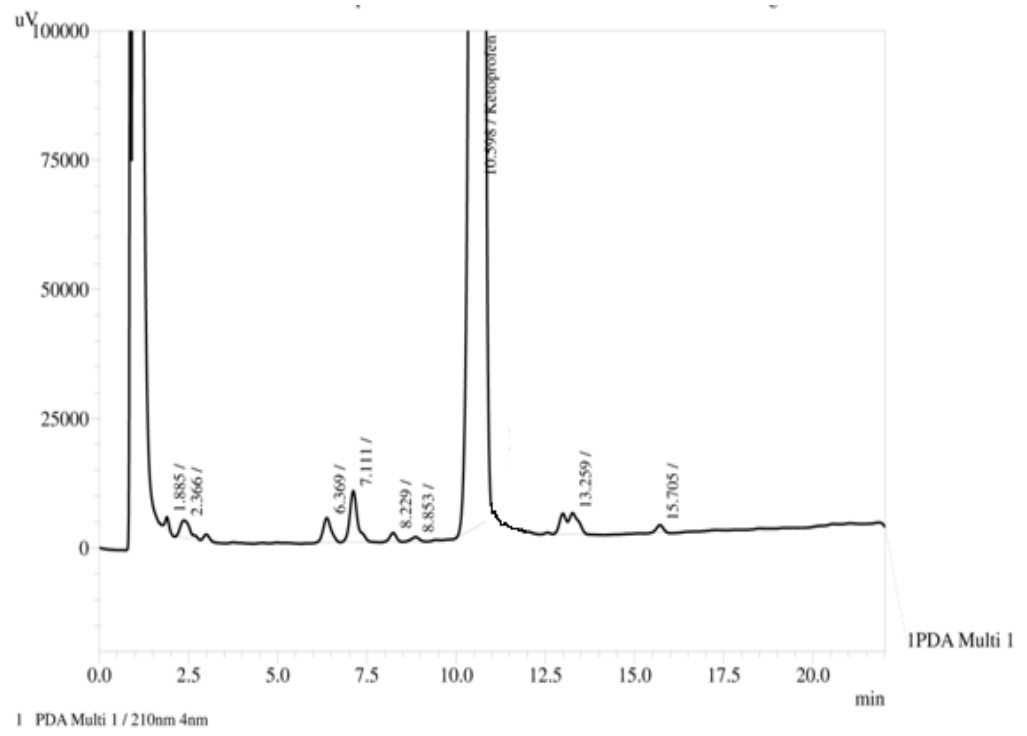

Fig 3. (B)

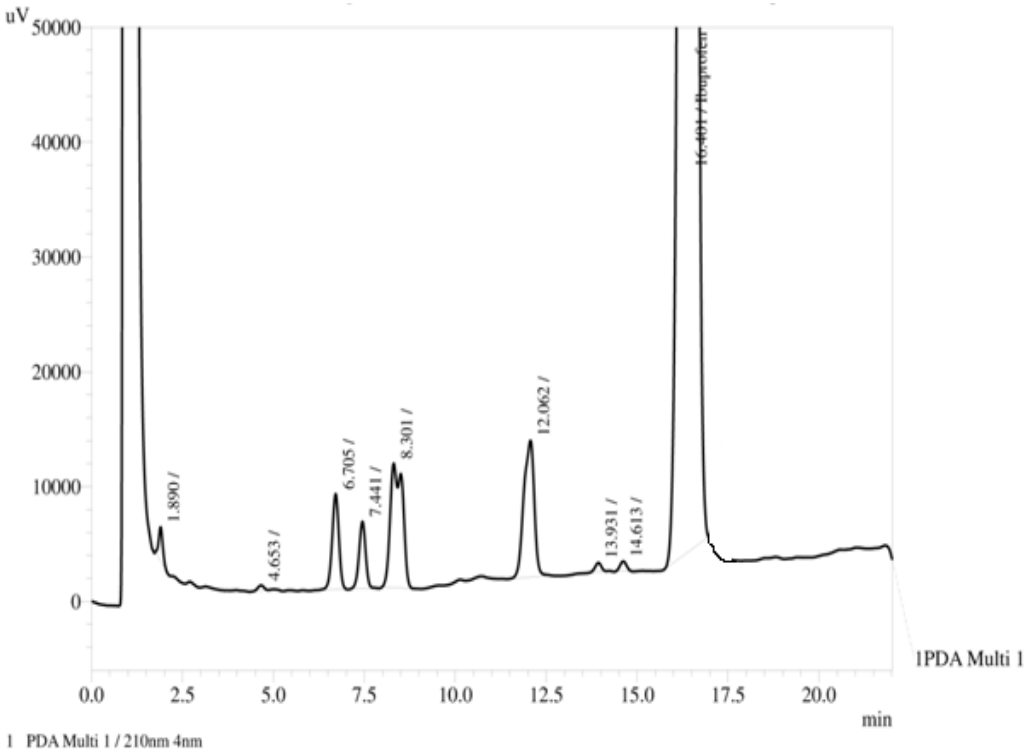

Fig 3. (C)

Fig. 3: HPLC chromatograms of 50 µL injection of 64 µg/mL HSC (A), 320 µg/mL KTP(B) and 640 µg/mL IBP (C) at 210 nm after oxidative degradation.

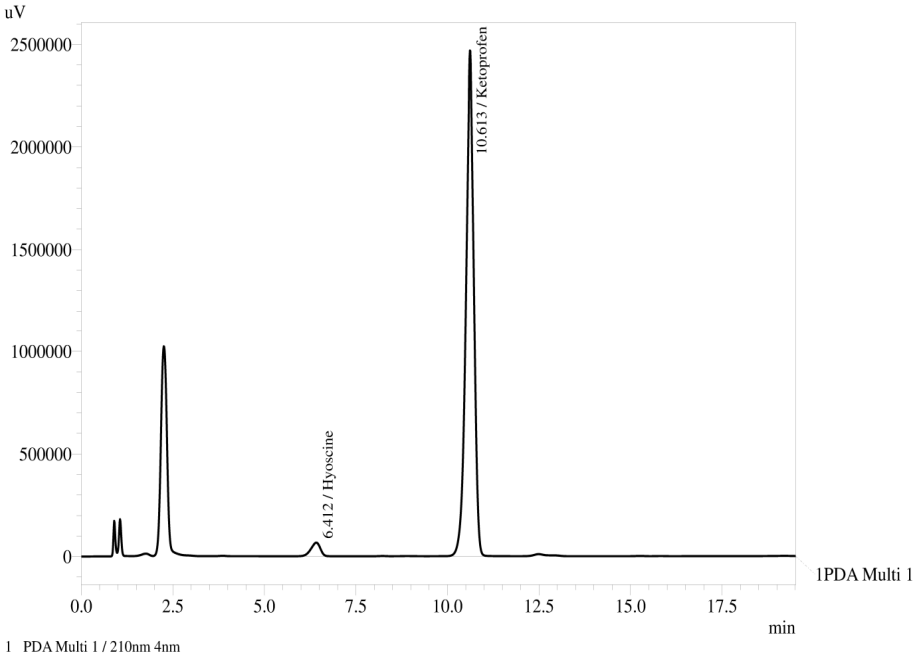

Fig. 4: HPLC chromatograms of 50 µL injection of a mixture containing 64 µg/mL HSC, and 320 µg/mL KTP obtained from spasmofen ampoules® at 210 nm.

Authors' Statement

The authors declare no conflict of interest.

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