

# Development and validation of highly selective method for the determination of imatinib mesylate and dexketoprofen trometamol combination in three different media

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Imatinib mesylate is a small molecule used in cancer therapy as a tyrosine kinase inhibitor. Dexketoprofen trometamol is a non-steroidal anti-inflammatory drug that has seen use in cancer therapy in combination with an anticancer drug to minimize tumor size and to reduce pain in patients. In the present study, imatinib mesylate and dexketoprofen trometamol were selected as potential model drugs to be used in combination. A new, simple and selective Ultra Performance Liquid Chromatography method was developed and validated to determine the drug substances in distilled water, in a pH 7.4 phosphate buffer and in Dulbecco's Modified Eagle Medium. The proposed method was developed using a BEH C-18 column with isocratic elution. A mixture of methanol:acetonitrile (80:20, v/v) and pH 9.5, 0.05 M ammonium acetate were (70:30, v/v) used as a mobile phase. Detection was carried out with a flow rate of 0.3 mL/min, a column temperature of 30°C and an injection volume of 20 µL. The method was validated considering linearity, accuracy, precision, specificity, robustness, detection limit and quantitation limit values, and was found to be linear in a range from 0.05 to 20.0 µg/mL for the three different media.

**Keywords:** Imatinib mesylate. Dexketoprofen trometamol. Ultra Performance Liquid Chromatography. Dulbecco's Modified Eagle Medium.

## INTRODUCTION

In pharmacy, toxicology and clinical analysis, the determination or assay of a drug substance has a great importance in terms of increasing productivity and reducing costs. Shortening the analysis time requires shortening column length, increasing flow rate, reducing the particle size of the column and increasing temperature. These approaches, however, come with various risks when applied to HPLC devices. For example, when a short and small particle sized column is used, the compounds in the sample may not always

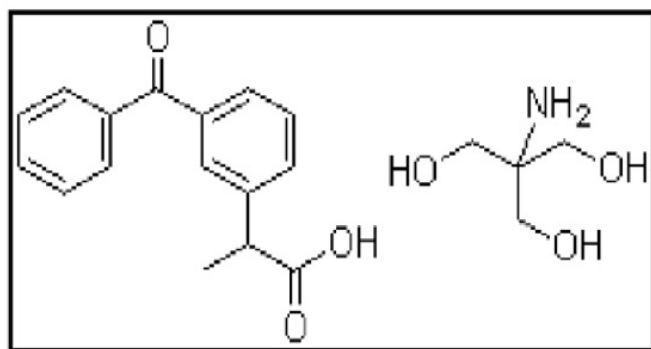
sufficiently separated, column efficiency may be reduced and high back pressure can be produced (Nováková, Solichová and Solich, 2006). Ultra Performance Liquid Chromatography (UPLC) was developed to overcome all these problems, allowing the separation of small molecules, leading to uniquely superior performance with significant improvements in resolution, sensitivity and speed (Antil *et al.*, 2013). In comparison with the HPLC method, it comes with a reduced analysis time and provides better separation (Gumustas *et al.*, 2013).

Dexketoprofen trometamol (DEX) is a water-soluble salt form of dextrorotatory and an *S*(+) enantiomer of ketoprofen, which is a non-steroidal anti-inflammatory drug (NSAID). It has been used as a non-selective cyclooxygenase (COX) enzyme inhibitor (Miranda *et al.*, 2011; Herrero *et al.*, 2003). Racemic ketoprofen is also

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used for analgesic and anti-inflammatory effects (Figure 1), although this effect can be observed mainly due to dexketoprofen, as the *R*(-) enantiomer is not associated with any such activity (Miranda *et al.*, 2011). The free acid form is almost insoluble in water, but soluble in organic solvents such as chloroform, ether and ethanol, while the trometamine salt is freely soluble in water as well as in organic solvents (Chandrasekharan, 2007).

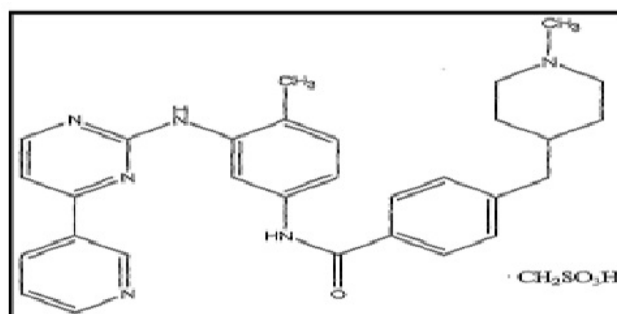
Imatinib mesylate (IMA) is a potent and selective inhibitor of protein tyrosine kinase Bcr-Alb, platelet-derived growth factor and c-KIT (Figure 2). The drug was approved by the FDA for the treatment of chronic myelogenous leukemia and gastrointestinal stromal tumors in 2001 and 2002 (FDA Consum. 2001; FDA Consum. 2002), respectively. In addition, it has started to be used for the treatment of different types of cancer (Ma, Xu, Shou, 2009; Cohen, Moses, Pazdur, 2002). For example, the dermatofibrosarcoma protuberans, aggressive fibromatoses, malignant melanoma and chordoma forms of cancer have all been treated with IMA (Iqbal, Iqbal, 2014). The active ingredient is an off-white to yellowish crystalline powder that is highly soluble in water, although the solubility of the drug is dependent on pH, with solubility decreasing with increasing pH (Novartis Pharmaceutical Corporation, 2017).



**FIGURE 1** - The chemical structure of dexketoprofen trometamol.

Epidemiological studies have indicated that the use of NSAIDs reduces the risk of the development of such fatal cancers as colorectal cancer. Since the COX-2 enzyme is responsible for increased prostaglandin production in inflammatory and neoplastic tissues, it continues to affect COX-2 during the treatment of cancer (Dannenberg *et al.*, 2001). It has also been observed that IMA increases COX-2 expression, and as a result, the

development of resistance to IMA over time has been reported as possible (Atari-Hajipirloo *et al.*, 2016). Recent studies have shown that the COX enzyme has become a target for the prevention of cancer. Many studies related to the use of combinations of NSAIDs with anticancer drugs can be found in literature as a new approach to the treatment of cancer (Liu *et al.*, 2017; Pan *et al.*, 2007; Mohammed *et al.*, 2003). Due to the positive results obtained from cell culture studies, dosage forms such as liposome and polymeric nanoparticles containing anticancer drug and NSAID in combination have also been developed (Gowda *et al.*, 2017; Singh, 2018).



**FIGURE 2** - The chemical structure of imatinib mesylate.

Various characterization studies into such topics as encapsulation efficiency, *in vitro* dissolution rate and *ex vivo* cell diffusion have been carried out on the developed formulations. The amount of drug in the formulation or releasing the drug to the medium need be calculated (Yue *et al.*, 2009; Miao *et al.*, 2015; Mutlu-A ardan *et al.*, 2016). For this reason, an analytical method specific to the drug should be developed for different media, such as distilled water, a pH 7.4 phosphate buffer, which seen use in dissolution studies, and Dulbecco's Modified Eagle Medium (DMEM), which has been used as a medium in cell culture studies. These media are preferred in the present study due to their recent use in the literature, especially in drug development studies. Distilled water was used as a dissolution medium in *in vitro* dissolution studies during the formulation development period. The pH 7.4 phosphate buffer is another frequently used dissolution medium. In formulation development studies, cell culture studies are particularly important, and DMEM has been also seen frequent use as a cell culture medium in such studies (Kumari *et al.*, 2018; Reed *et al.*, 2012). In previous literature, many liquid chromatography methods for

IMA and DEX are available in several media, although there is as yet no chromatographic method for the simultaneous determination of IMA and DEX (Ivanovic *et al.*, 2004; Moreno *et al.*, 2013; Archana, Vikas, 2013; İlbasımış-Tamer, 2017).

Combinations of anticancer drugs and NSAID have proven to be superior to monotherapies, and so there is an apparent need in the future for a proper method of analysis for these drugs in various media in combination for formulation development, quality control or other purposes. IMA has been used in cancer therapy and DEX is a potent NSAID, and it is clear that a combination of the two will be formulated soon. In present study, DEX, which is an NSAID, and IMA, which is an anticancer agent, have been chosen as the model drugs in an attempt to develop a UPLC method for the simultaneous analysis of these drugs in various media. For the present study, three different media were used: distilled water (for encapsulation efficiency), pH 7.4 phosphate buffer (for an *in vitro* release study) and DMEM (for an *ex vivo* release study).

## MATERIAL AND METHODS

### Material

Analytical grade methanol ( $\geq 99.8\%$ , Sigma, Germany), acetonitrile ( $\geq 99.93\%$ , Sigma, Germany), an ammonium hydroxide solution ( $\geq 25\text{-}30\%$ , Sigma, Germany) and ammonium acetate (Merck, Germany) were used in this study for the preparation of the mobile phase. The IMA was supplied by Biotang (USA); the DEX and potassium dihydrogen phosphate (99.995%) were purchased from Sigma-Aldrich (Germany); the DMEM was supplied from Lonza (Belgium); and the Bi-distilled water was prepared using a Barnstead water purification system (Barnstead, USA).

### Analytical and chromatographic conditions

A UPLC system (Waters Acquity UPLC) with Empower software, equipped with a binary solvent pump, a photo diode array detector and BEH C-18 column (100x2.1 mm, 1.7  $\mu\text{m}$  particle size, Acquity, Waters, Ireland) were used. An ultrasonic bath was used for the degassing of the solvent systems. A variety of mobile phases were tested during the development stage of the method for the simultaneous analysis of DEX and IMA. A mixture of methanol:acetonitrile (80:20, v/v)

and 0.05 M ammonium acetate (pH 9.5 adjusted with ammonium hydroxide) (70:30, v/v) was found to be the most suitable mobile phase. The flow rate of the mobile phase and the injection volume of the sample were set to 0.3 mL/min and 20  $\mu\text{L}$ , respectively. The column temperature was maintained at 30 °C. The detection of the drug was carried out at 258 nm for the distilled water and pH 7.4 phosphate buffer media, and 326 nm for DMEM. The total run time was 3 minutes.

### Preparation of mobile phase, stock solution and samples

For the preparation of the mobile phase, ammonium acetate (0.964 g) was dissolved in 250 mL distilled water in a volumetric flask and the pH of the solution was adjusted to 9.5 with ammonium hydroxide.

Stock solutions and samples were prepared as follows: DEX (10 mg) and IMA (10 mg) were transferred into volumetric flask and filled up to 100 mL with distilled water, along with the pH 7.4 phosphate buffer or DMEM. The flask was stirred until all ingredients were dissolved. The final concentration of the stock solution was 100  $\mu\text{g}/\text{mL}$ . Different volumes of the stock solution were transferred to other 10 mL volumetric flasks and filled up to the desired volumes with the media. The mobile phase and all other solutions were filtered through a filter paper (0.22  $\mu\text{m}$  pore size) prior to the analysis. In this way, calibration samples were obtained with a concentration range of 0.05 to 20  $\mu\text{g}/\text{mL}$ . The calibration curves were then obtained by plotting the peak area against the concentration of drug. Each concentration was analyzed in six replicates.

### Validation of the analytical method

The validation studies were carried out based on the International Conference on Harmonisation (ICH) Validation of Analytical Procedures: Text and methodology Q2(R1). The validation parameters, which were indicated in the guideline, were linearity, accuracy, precision (repeatability and intermediate precision), specificity, robustness, detection limit and quantitation limit (ICH, 2005).

## RESULTS AND DISCUSSION

The UPLC proposed in the present study is new, and is appropriate for the simultaneous determination of

DEX and IMA. The novelty of the method was confirmed through a detailed literature search, which revealed no studies of such a simultaneous determination. The search did, however, reveal some HPLC and UPLC methods for the individual analyses of DEX or IMA in a phosphate buffer or plasma (Rezende, 2013; Sahoo, 2015), while no method was identified in literature for DMEM for these substances. For instance, one HPLC method that was successfully validated was found to be useful for the measurement of IMA concentrations in samples from chronic myeloid leukemia patients to check for treatment compliance (Rezende, 2013). Other methods were reported to be useful and being validated in literature for DEX. Although a few successfully validated methods have been published for individual analyses of DEX or IMA, none were found to be directly comparable with the method proposed in the present study (İlbasmış-Tamer, 2017; Mulla, 2011).

### Optimization of chromatographic conditions

To achieve optimal chromatographic separation, different mobile phases and flow rates were tested. Various buffer systems and mobile phases (e.g. potassium dihydrogen phosphate buffers with different molarity, formic acid of different percentages in water or methanol, and various organic solvent systems with different pH values) were also used to develop the analysis method for IMA and DEX. However, the symmetric and sharp peaks of IMA at the initial part of UPLC method development studies could not be obtained. According to the study by Nageswari, Reddy and Mukkanti (2012), reported chromatographic conditions were modified. The proposed method was developed using a BEH C-18 column with isocratic elutions. A mixture of methanol:acetonitrile (80:20, v/v) and pH 9.5, 0.05 M ammonium acetate was used in a 70:30 v/v ratio as a mobile phase. Various mobile phases were used to obtain a good separation. For example, various buffer systems and mixtures of organic solvents, such as methanol/acetonitrile with water at various ratios, were tested, but the most successful and satisfactory results were obtained with a pH 9.5 acetate buffer. The chromatographic conditions were a flow rate of 0.3 mL/min, a column temperature of 30 °C and an injection volume of 20 µL. As a result, for both IMA and DEX, smooth, sharp and symmetrical peaks were obtained. Validation studies were performed on an Agilent Waters UPLC

system with Empower software, and the method was validated according to ICH(Q2) guidelines (linearity, accuracy, precision, specificity and robustness, limit of detection (LOD) and limit of quantification (LOQ) values were calculated).

### Parameters of validation

In line with the method mentioned above, six parallel studies were carried out for IMA and DEX for each medium, with linearity evaluated first. Samples with concentrations ranging from 0.05 to 20 µg/mL were prepared, and the peak area of drug substances corresponding to the concentration of drug substances was plotted (Figure 3, Figure 4 and Figure 5).

The accuracy of a measurement system is defined as the degree of closeness of the measurements to the real value. Samples with known concentrations were analyzed for accuracy with three working level concentrations. Accordingly, 80, 100 and 120% of the expected concentrations of analyte in the sample was calculated separately for each medium (Table I and Table II).

Precision is related to reproducibility and repeatability, which can be calculated after repeated measurements under unchanging conditions. System precision is evaluated from standard deviation (SD) and relative standard deviation (RSD). Intra-day precision the samples were prepared (10 µg/mL) and injected into system 10 times, while inter-day precision was determined by analyzing the same samples on three different days. The results of the intra-day and inter-day precisions are presented in the tables (Table III, Table IV, Table V and Table VI).

The applied method must detect only the desired drug substance, both specifically and selectively. In a tested formulation, other substances should not give any peak at the same retention time, and this must be checked. The developed method was shown to be specific for IMA and DEX, since the peaks of drug substance were observed on the chromatogram at different retention times (Figure 6, Figure 7 and Figure 8) and no interference was observed from any other substance.

For the developed analytical method, robustness studies should also be performed to ensure that measurements are not affected by minor experimental changes. For example, the stability of analytical solutions, extraction time, pH value of the mobile phase, mobile phase component ratios or column can be considered

as minor variations. In this study, the stability of the analytical solutions at various temperatures and times were investigated by calculating the percentage of the

remaining DEX and IMA (after 72 hours at 4, 25 and 37 °C). There was no significant differences  $p > 0.05$  (Table VII).

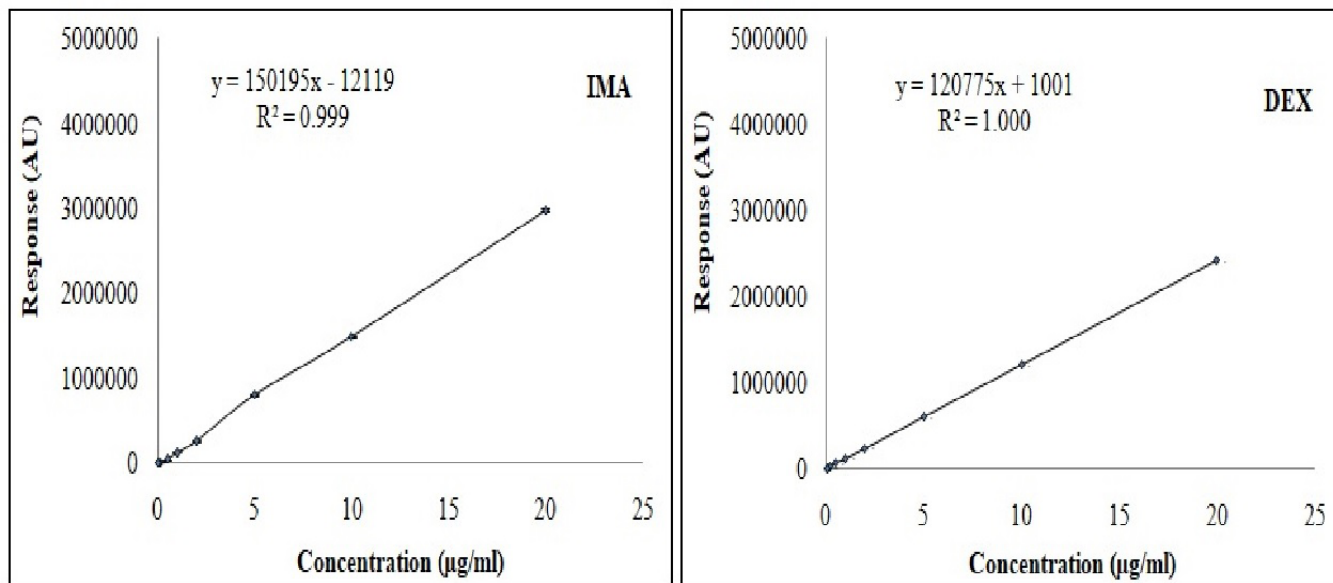


FIGURE 3 - The calibration curves of imatinib mesylate and dexketoprofen trometamol in distilled water (n=6).

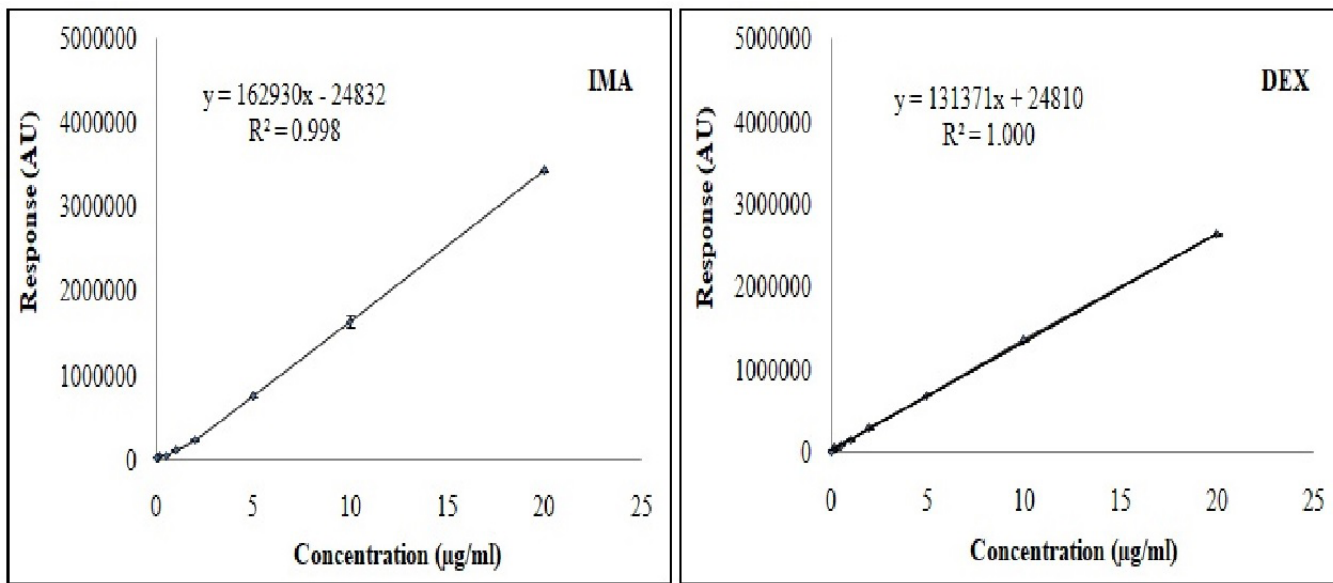
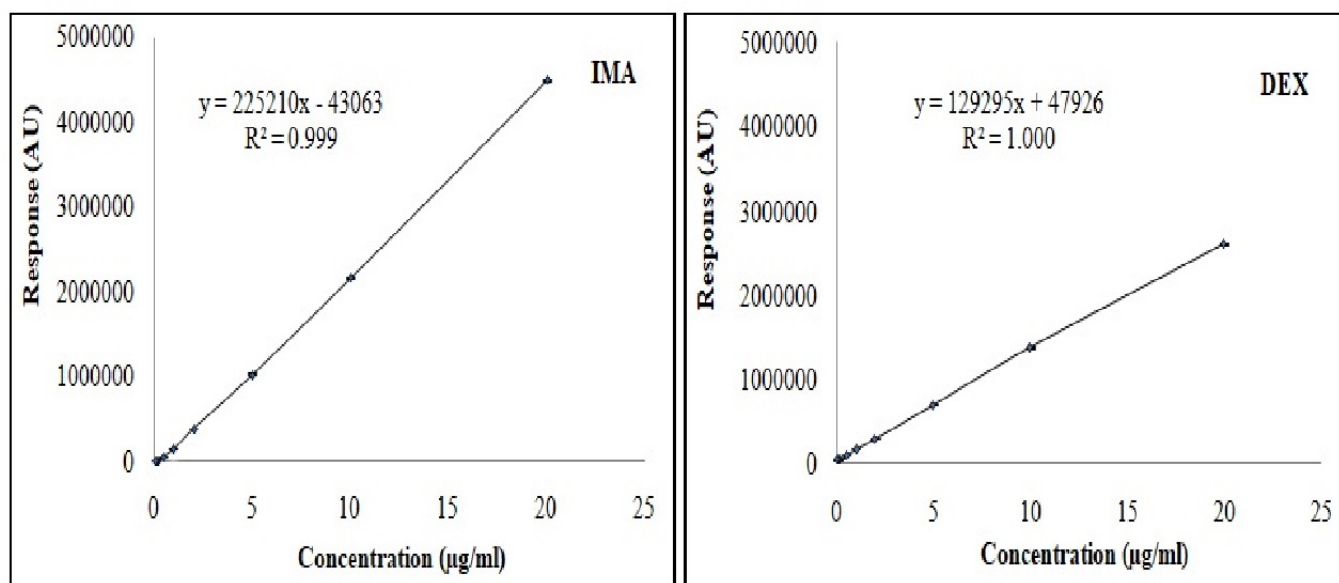


FIGURE 4 - The calibration curves of imatinib mesylate and dexketoprofen trometamol in phosphate buffer (n=6).



**FIGURE 5** - The calibration curves of imatinib mesylate and dexketoprofen trometamol in DMEM (n= 6).

**TABLE I** - Accuracy and recovery report of imatinib mesylate

	Percentage level (%)	Concentration of IMA in solution (µg/mL)	Calculated concentration of IMA (µg/mL) Mean±SD	RSD%
Distilled water	120	12	12.100±0.029	0.239
	100	10	10.040±0.006	0.058
	80	8	8.140±0.031	0.386
pH 7.4 Phosphate Buffer	120	12	12.434±0.077	0.619
	100	10	10.291±0.027	0.263
	80	8	8.055±0.023	0.284
DMEM	120	12	12.004±0.050	0.414
	100	10	9.999±0.002	0.021
	80	8	7.998±0.019	0.236

IMA is imatinib mesylate. Conc. is concentration. DMEM is Dulbecco’s Modified Eagle Medium. SD is standard deviation and RSD is relative standard deviation (n=3).

**TABLE II** - Accuracy and recovery report of dexketoprofen trometamol

	Percentage level (%)	Concentration of DEX in solution ( $\mu\text{g/mL}$ )	Calculated concentration of DEX ( $\mu\text{g/mL}$ ) Mean $\pm$ SD	RSD%
Distilled water	120	12	11.911 $\pm$ 0.007	0.061
	100	10	10.012 $\pm$ 0.014	0.136
	80	8	8.032 $\pm$ 0.007	0.090
pH 7.4 Phosphate Buffer	120	12	12.213 $\pm$ 0.015	0.120
	100	10	10.071 $\pm$ 0.007	0.071
	80	8	7.932 $\pm$ 0.004	0.050
DMEM	120	12	12.263 $\pm$ 0.093	0.757
	100	10	10.166 $\pm$ 0.080	0.785
	80	8	8.070 $\pm$ 0.056	0.694

DEX is dexketoprofen trometamol. Conc. is concentration. DMEM is Dulbecco's Modified Eagle Medium. SD is standard deviation and RSD is relative standard deviation (n=3).

**TABLE III** - Intra-day precision for imatinib mesylate

Distilled water		pH 7.4 Phosphate Buffer		DMEM	
Concentration ( $\mu\text{g/mL}$ )	Analyzed Value	Concentration ( $\mu\text{g/mL}$ )	Analyzed Value	Concentration ( $\mu\text{g/mL}$ )	Analyzed Value
20	20.067	20	20.159	20	19.949
20	20.088	20	20.120	20	19.951
20	20.020	20	20.129	20	19.949
20	20.090	20	20.127	20	19.948
20	20.014	20	20.139	20	19.946
20	20.029	20	20.124	20	19.943
20	20.010	20	20.133	20	19.934
20	20.088	20	20.130	20	19.943

(continuing)

**TABLE III** - Intra-day precision for imatinib mesylate

Distilled water		pH 7.4 Phosphate Buffer		DMEM	
Concentration (µg/mL)	Analyzed Value	Concentration (µg/mL)	Analyzed Value	Concentration (µg/mL)	Analyzed Value
20	20.013	20	20.095	20	19.926
20	20.071	20	20.028	20	19.914
Mean	20.049	Mean	20.118	Mean	19.940
SD	0.202	SD	0.036	SD	0.120
RSD%	0.981	RSD%	0.177	RSD%	0.619

DMEM is Dulbecco's Modified Eagle Medium. SD is standard deviation and RSD is relative standard deviation (n=10).

**TABLE IV** - Intra-day precision for dexketoprofen trometamol

Distilled water		pH 7.4 Phosphate Buffer		DMEM	
Concentration (µg/mL)	Analyzed Value	Concentration (µg/mL)	Analyzed Value	Concentration (µg/mL)	Analyzed Value
10	10.011	10	10.135	10	10.445
10	10.021	10	10.128	10	10.448
10	10.034	10	10.125	10	10.433
10	10.027	10	10.124	10	10.433
10	10.018	10	10.143	10	10.426
10	10.033	10	10.127	10	10.420
10	10.036	10	10.135	10	10.407
10	10.040	10	10.141	10	10.397
10	10.048	10	10.136	10	10.394
10	10.038	10	10.136	10	10.386
Mean	10.031	Mean	10.133	Mean	10.419
SD	0.011	SD	0.007	SD	0.022
RSD%	0.112	RSD%	0.066	RSD%	0.216

DMEM is Dulbecco's Modified Eagle Medium. SD is standard deviation and RSD is relative standard deviation (n=10).



**TABLE V** - Inter-day precision for imatinib mesylate

	Concentration ( $\mu\text{g/mL}$ )	1.day	2. day	3. day	Mean	SD	RSD%
Distilled water	20	19.977	19.975	19.968	19.974	0.047	0.239
	5	5.015	5.014	5.004	5.011	0.059	1.147
	1	0.994	0.996	0.996	0.996	0.010	1.069
	0.2	0.196	0.196	0.196	0.196	0.001	0.923
pH 7.4 Phosphate Buffer	20	19.964	20.069	19.899	19.977	0.086	0.430
	5	4.834	4.900	4.929	4.888	0.049	0.993
	1	0.944	0.945	0.924	0.938	0.012	1.275
	0.2	0.216	0.221	0.218	0.218	0.003	1.266
DMEM	20	20.019	20.019	20.023	20.020	0.026	0.127
	5	4.979	4.979	4.978	4.979	0.001	0.107
	1	0.997	0.997	0.997	0.997	0.001	0.156
	0.2	0.204	0.204	0.204	0.204	0.002	0.098

DMEM is Dulbecco's Modified Eagle Medium. SD is standard deviation and RSD is relative standard deviation (n=3).

**TABLE VI** - Inter-day precision for dexketoprofen trometamol

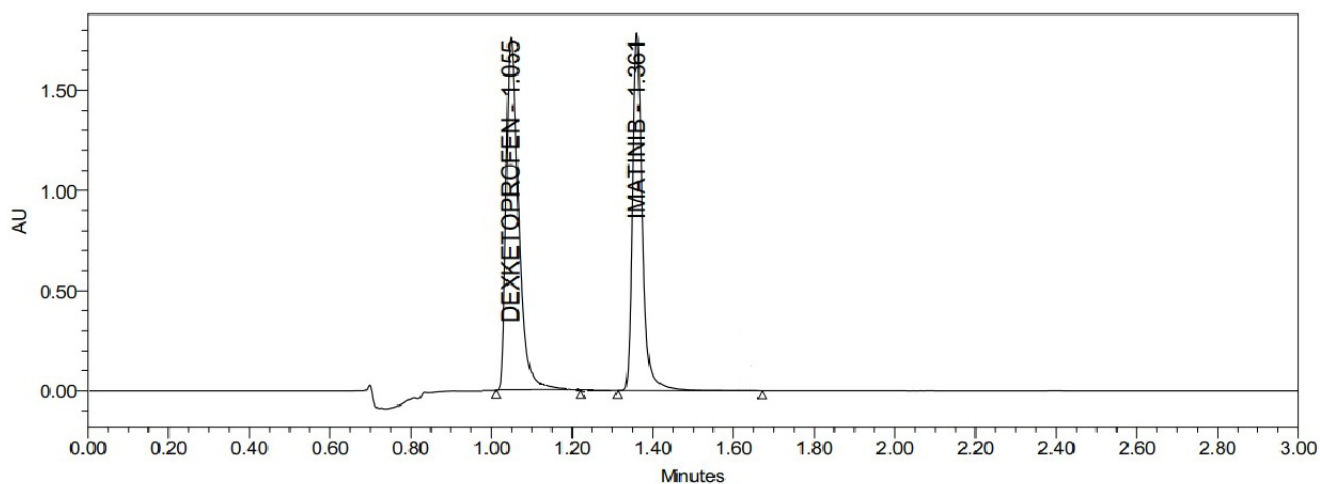
	Concentration ( $\mu\text{g/mL}$ )	1.day	2. day	3. day	Mean	SD	RSD%
Distilled water	10	10.034	10.046	10.024	10.035	0.011	0.110
	2	1.963	1.965	1.963	1.964	0.001	0.065
	0.5	0.487	0.487	0.488	0.487	0.001	0.185
	0.09	0.087	0.087	0.086	0.087	0.000	0.381
pH 7.4 Phosphate Buffer	10	9.947	9.890	9.840	9.892	0.053	0.541
	2	2.018	2.004	2.011	2.011	0.007	0.358
	0.5	0.499	0.503	0.496	0.499	0.004	0.713
	0.1	0.094	0.094	0.093	0.093	0.001	0.804

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**TABLE VI** - Inter-day precision for dexketoprofen trometamol

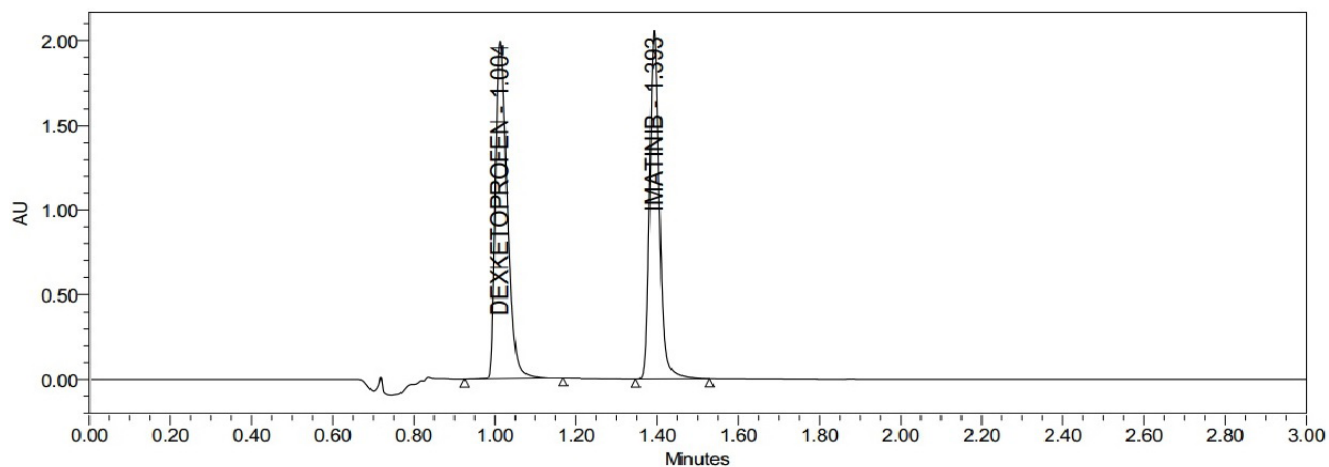
	Concentration ( $\mu\text{g/mL}$ )	1.day	2. day	3. day	Mean	SD	RSD%
DMEM	10	10.254	10.263	10.085	10.201	0.100	0.984
	2	1.984	2.024	2.002	2.004	0.020	0.999
	0.5	0.465	0.468	0.477	0.470	0.006	1.306
	0.05	0.051	0.051	0.051	0.051	0.000	0.821

DMEM is Dulbecco's Modified Eagle Medium. SD is standard deviation and RSD is relative standard deviation (n=3).



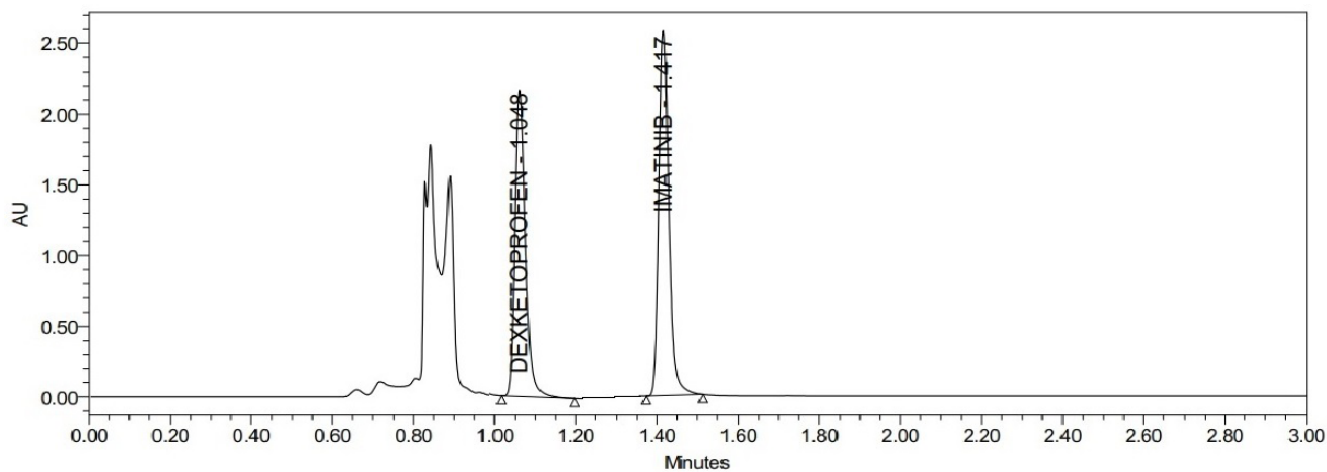
	Peak Name	RT	Area	%Area	Height	Units
1	DEXKETOPROFEN	1.055	2418640	100.00	1758477	ug/ml
2	IMATINIB	1.361	3021155	100.00	1785142	ug/ml

**FIGURE 6** - UPLC chromatogram of dexketoprofen trometamol and imatinib mesylate in distilled water.



	Peak Name	RT	Area	%Area	Height	Units
1	DEXKETOPROFEN	1.004	2639684	100.00	1936308	ug/ml
2	IMATINIB	1.393	3448071	100.00	2057061	ug/ml

**FIGURE 7** - UPLC chromatogram of dexketoprofen trometamol and imatinib mesylate in phosphate buffer.



	Peak Name	RT	Area	%Area	Height	Units
1	DEXKETOPROFEN	1.048	2635628	100.00	2140333	ug/ml
2	IMATINIB	1.417	4503339	100.00	2580517	ug/ml

**FIGURE 8** - UPLC chromatogram of dexketoprofen trometamol and imatinib mesylate in DMEM.

**TABLE VII** - P-values of remained DEX and IMA in solutions for robustness

	Distilled water			pH 7.4 Phosphate Buffer			DMEM		
	4 °C	25 °C	37 °C	4 °C	25 °C	37 °C	4 °C	25 °C	37 °C
IMA	0.909	0.076	0.065	0.391	0.391	0.103	0.423	0.085	0.126
DEX	0.215	0.080	0.066	0.441	0.431	0.080	0.415	0.078	0.113

P > 0.05, level of significance.

LOD is the lowest quantity of analyte in a sample that can be detected, but not certainly quantified while LOQ is the lowest quantity of analyte in a sample in which the quantity of analyte can be determined as an absolute value. Both can be calculated based on the standard deviation of the response and the slope (ICH, 2005). The results are presented in Table VIII and Table IX.

The validation parameters were obtained from various repeated studies (number of replicates shown in brackets for each result). The results were given as mean±standard deviation, and the calibration curve of the analytical method was obtained considering concentrations versus peak areas. A quite high correlation was obtained ( $r^2:0.999$  for both of samples in each medium) with the concentration range of 0.05–20 µg/mL in the three different media for DEX and IMA. The concentrations of 10 µg/mL DEX and IMA were used to test the accuracy of the method, for which 80% and 120% concentrations were prepared, and all samples were analyzed. The amounts of DEX and IMA in these solutions were determined using the proposed method, and the accuracy of the methods was found to be within the acceptable range. System precision was calculated also for the ten samples, while intermediate precision was determined by analyzing the samples on different days. The precision values were found to be suitable. A simultaneous analysis of IMA and DEX showed that the method is specific for both drug substances, in that the peaks of the analytes were observed at different retention times, and these peaks were far away from the peak of the solvent on the chromatogram. In the robustness studies, the samples were kept at various temperatures and compared with the initial values, and no significant difference was observed between the groups ( $P > 0.05$ ). The LOQ and LOD for DEX and IMA were found to be suitable. For all parameters, RSD values were lower than 2%.

**TABLE VIII** - Limit of detection and limit of quantitation values for imatinib mesylate

	Distilled water	pH 7.4 Phosphate Buffer	DMEM
LOQ (µg/mL)	0.014	0.037	0.012
LOD (µg/mL)	0.005	0.012	0.004

DMEM is Dulbecco's Modified Eagle Medium. LOQ is limit of quantification. LOD is limit of detection.

**TABLE IX** - Limit of detection and limit of quantitation values for dexketoprofen trometamol

	Distilled water	pH 7.4 Phosphate Buffer	DMEM
LOQ (µg/mL)	0.006	0.013	0.038
LOD (µg/mL)	0.002	0.004	0.013

DMEM is Dulbecco's Modified Eagle Medium. LOQ is limit of quantification. LOD is limit of detection.

## CONCLUSION

A rapid, isocratic UPLC method was developed for quantitative simultaneous analysis of DEX and IMA. The method was found to be precise, accurate, linear and fast. This method may be useful for researchers interested in carrying out *in vitro* and *ex vivo* studies,

such as quantification, dissolution, stability and cell culture studies, with IMA, DEX, or a combination of the two.

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