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ORIGINAL ARTICLE

Development and Validation of an Ultra Performance Liquid Chromatography Method for the Determination of Dexketoprofen Trometamol, Salicylic Acid and Diclofenac Sodium

Deksketoprofen Trometamol, Salisilik Asit ve Diklofenak Sodyum Etkin Maddeleri için Ultra Performanslı Sıvı Kromatografisi Yönteminin Geliştirilmesi ve Validasyonu

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ABSTRACT

Objectives: A simple, fast, accurate and precise method has been developed for the determination of dexketoprofen trometamol (DKP), salicylic acid (SA) and diclofenac sodium (DIC) in the drug solutions using ultra high performance liquid chromatography (UPLC).

Materials and Methods: UPLC method is highly reliable and sensitive method to quantify the amount of the active ingredient and the method is validated according to ICH guidelines.

Results: The developed method is found to be precise, accurate, specific and selective. The method was also found to be linear and reproducible. The value of limit of dedection (LOD) of DKP, SA, DIC were found 0.00325 µg/mL, 0.0027 µg/mL and 0.0304 µg/mL, respectively. The limit of quantitation (LOQ) of DKP, SA and DIC were found 0.00985 µg/mL, 0.0081 µg/mL and 0.0920 µg/mL, respectively.

Conclusion: Proposed methods can be successfully applicable to the pharmaceutical preparation containing the above mentioned drugs (dexketoprofen trometamol, salicylic acid and diclofenac sodium). Even very small amounts of active substance can be analyzed and validations can be performed easily.

Key words: Dexketoprofen trometamol, salicylic acid, diclofenac sodium, UPLC, validation

ÖZ

Amaç: Deksketoprofen trometamol (DKP), salisilik asit (SA) ve diklofenak sodyumun (DIC) ilaç çözeltisindeki analizi için ultra yüksek basınçlı sıvı kromatografisi (UPLC) kullanılarak basit, hızlı, doğru ve kesin bir yöntem geliştirilmiştir.

Gereç ve Yöntemler: UPLC yöntemi aktif bileşikleri analiz etmek için oldukça hassas bir yöntemdir ve yöntem ICH kurallarına göre valide edilmiştir.

Bulgular: Geliştirilen yöntem, kesin, doğru, spesifik ve seçici bulunmuştur. Yöntem, doğrusal ve tekrarlanabilir bulunmuştur. DKP maddesi için teşhis sınırı-duyarlılık sınırı (LOD) 0.00325 µg/mL ve tayin alt sınırı-saptama sınırı (LOQ) 0.00985 µg/mL olarak bulunmuştur. SA için LOD 0.0027 µg/mL ve LOQ 0.0081 µg/mL olarak bulunmuştur. DIC için LOD 0.0304 µg/mL ve LOQ 0.0920 µg/mL olarak bulunmuştur.

Sonuç: Böylece önerilen yöntemler yukarıda bahsedilen ilaçları içeren (deksketoprofen trometamol, salisilik asit ve diklofenak sodyum) farmasötik preparatlarda başarılı bir şekilde uygulanabilecektir. Aktif bileşikler çok küçük miktarlarda analiz edilebilecek ve kolaylıkla valide edilebilecektir.

Anahtar kelimeler: Deksketoprofen trometamol, salisilik asit, diklofenak sodyum, UPLC, validasyon

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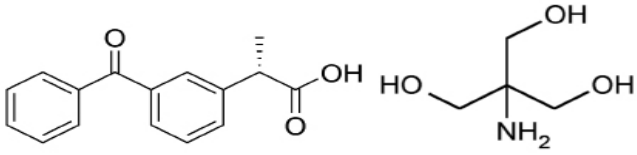
INTRODUCTION

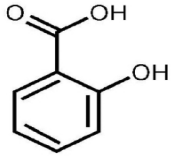
Dexketoprofen trometamol (DKP) chemically, 2-amino-2-(hydroxymethyl) propane-1,3-diol; 2-(3-benzoylphenyl propionic acid is a water-soluble salt of the (S)-(+)- enantiomer of the non-steroidal anti-inflammatory drug (NSAID) ketoprofen.[1] The enantiomer is a relatively new oral NSAID with analgesic, anti-inflammatory and anti-pyretic properties and is one of the most potent in vitro inhibitors of prostaglandin synthesis.[2] DKP is a new, quick acting analgesic for the treatment of painful musculoskeletal conditions such as osteoarthritis and low back pain. It is also used as a treatment for post-operative pain, toothache and dysmenorrhea.[3] It is the active optical isomer (eutomer) of ketoprofen, a propionic acid NSAID. The eutomer has been separated to halve the dosage required and halve the metabolic load. The inactive isomer (distomer) has been discarded in the hope of eliminating or reducing potential unnecessary side effects[4] (Graphic 1).



Dexketoprofen trometamol[5]

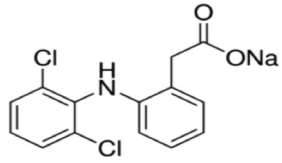
Salicylic acid (SA) is aminohydroxybenzoic acid, a type of phenolic acid and a beta hydroxy acid. It has the formula $C_7H_6O_3$. SA is the most widely consumed analgesic, antipyretic, and anti-inflammatory agent in the World.[6] It is a natural product found in the bark of a willow tree and has been used to relieve fever and pain.[7] SA is a precursor to acetylsalicylic acid, better known as aspirin.[8] SA is used topically for its keratolytic, bacteriostatic, fungicidal, and photoprotective properties. Topical application has been shown to reduce the rate of keratinocyte proliferation. It also inhibits cholesterol sulfotransferase, an enzyme responsible for cholesterol sulfate formation within keratinocytes. SA directly solubilizes the stratum corneum by dissolving the intercellular cement. Through these mechanisms, SA increases the elimination of squames from the stratum corneum.[9] The principal use of topical SA in dermatology is as a keratolytic agent. SA toxicity can occur with topical use of 6% SA over as little as 40% body surface area.[10]

Diclofenac (DIC) sodium is chemically 2-[2-(2,6-dichlorophenylamino)phenyl] acetic acid, a NSAID exhibits anti-inflammatory and analgesic properties. The primary mechanism responsible for its anti-inflammatory, antipyretic, and analgesic action is thought to be inhibition of prostaglandin synthesis by inhibition of cyclooxygenase (Graphic 2).



Salicylic acid[8]

It also appears to exhibit bacteriostatic activity by inhibiting bacterial DNA synthesis[11] (Graphic 3).



Diclofenac sodium[12]

The chosen data from literature sources are shown in Table 1.[13,14,15] It is well known that DKP is highly soluble in water and class 1 group compound according to the biopharmaceutical classification system. Permeability is also high.[16] SA is class 1 compound, and highly soluble in water.[17] DIC has poor water solubility and high permeability as class 2 compound.[18] The solubility, permeability properties, molecular weights, melting points, ionization constants and octanol-water partition coefficient of active ingredients are different from each other. The ultra performance liquid chromatography (UPLC) methods were developed of these different active ingredients.

Although several high-performance liquid chromatography methods can be found in the literature for DKP, SA and DIC to date, there is a few UPLC methods for aforementioned active ingredients. In the present investigation, a simple, optimized, and validated UPLC methods were proposed for the standardization of DKP, SA, DIC. The aim of this study was to develop and validate an analytical method for DKP, SA, DIC in buffer solution. Methods validation is the process of demonstrating that analytical procedures are suitable for their intended use, to ensure the identity, strength, quality, purity, and potency of the drug substance and drug product.[19,20] Linearity, accuracy, repeatability, specificity, sensitivity and detection limit parameters were validated by examining these parameters.

EXPERIMENTAL

Materials

DKP was purchased Huangshi Shixing Pharmaceutical Co. Ltd. (China). SA was purchased from Botafarma Pharmaceutical Laboratory (Turkey) and DIC was obtained from Fako Pharmaceuticals, Inc. (Düzce, Turkey).

Acetonitrile, methanol, ammonium chloride, monosodium phosphate and disodium phosphate were purchased fom Merck Darmstadt (Germany). Acetone was purchased from Sigma-aldrich, MO, (USA), potassium dihydrogen phosphate was purchased from Reidel-deHaën, (Germany), distilled water (18 MW.cm).

Instrumentation

Waters Acquity, UPLC system was used (MA, USA). Inertsil® (ODS-4, GL Sciences, 2 µm, 2.1x50 mm, C/N 5020-81202, S/N OFF50005) was used as a UPLC colon. Diode array dedector was used.

Table 1. Used physicochemical properties of the active ingredients[13,14,15]

	Molecular weight (g/moL)	Topological polar surface area (A2)	Melting point (°C)	Log P (Oct/water)	Pka
Dexketoprofen	375.4	141	104	3.36	3.88
Salicylic acid	138	57.5	159	2.26	2.97
Diclofenac	318	52.2	275	4.40	3.80

Pka: Lonization constant

Ultra performance liquid chromatography assay method and validation of active ingredients

UPLC method was developed to quantify the drug in the saturated drug solutions. Active ingredients were dissolved in 25% pH 7.4 sodium phosphate buffer. To prepare phosphate buffer at pH 7.4; KH_2PO_4 (250 mL, 0.2 M) was prepared, then 0.2 M NaOH (195.5 mL) was mixed and completed to 1 liter with deionized water. Then, dilution was made with deionized water. The purpose of 25% diluting the buffer solution is to reduce ion concentration of solution thereby facilitating the analysis of the active substance. After dilution was checking whether there is change in pH. It was determined that the pH of the environment remains constant.

Ultra performance liquid chromatography assay method and validation of dexketoprofen trometamol

UPLC methods and conditions of DKP was adopted and validated.[2,4,5,21] The method was found to be linear and reproducible. The A solvent was acetonitrile and the B solvent was MeOH/Water (1/1) (v/v). Starting conditions were 98% B, and within two min 30% B was employed. The final condition is 98% B. The temperature was 25˚C. Ultraviolet (UV) absorbance data were collected at 254 nm. The flow rate was 0.25 mL/min. The retention time was 0.734 min. Injection volume was 20 µL.

Ultra performance liquid chromatography assay method and validation of salicylic acid

UPLC method of SA was also adopted from literature and validated.[22,23,24,25] The gradient mobile phase flow was almost same with dexketoprofen analysis. The temperature was 25˚C. UV absorbance data were collected at 292 nm. The flow rate was 0.25 mL/min. The retention time was 0.704 min. Injection volume was 20 µL.

Ultra performance liquid chromatography assay method and validation of diclofenac sodium

UPLC method and conditions for DIC was also adopted.[11,12,26,27] The A solvent was acetonitrile and B solvent was 50 mM acetate buffer (1/1), (v/v) pH 3.1. The column temperature was stable at 25˚C. UV absorbance data were collected at 254 nm. The flow rate was 0.5 mL/min. The retention time was 1.22 min. Injection volume was 20 µL. The method used was found to be reproducible. Injection volume was 20 µL.

Real sample applications

To evaluate the performance of the proposed method, real sample application was performed using commercial tablet, test tablet and commercial eye drop. Test tablets were prepared with SA, lactose, starch 1500, magnesium stearate, aerosil 200. The brand name of commercial DKP tablet is Arveles® 25 mg film tablets. The brand name of commercial DIC eye drop is Inflased® 1%, 5 mL. In this scope, the amounts of DKP, SA and DIC were determined in commercial tablet, test tablet and eye drop, respectively.

Ten tablets (each tablet containing 36.9 mg DKP) were weighed and finally powdered. A portion of powder equivalent to about 36.9 mg DKP was weighed accurately and dissolved completely in exact volume of 369 mL phosphate buffered saline (PBS). Then, solution was stirred for 154 min on a magnetic stirrer. The solution was filtered and diluted with PBS up to mark. 20 µL volume of sample solution was injected into the column.

Ten tablets (each tablet containing 1% SA) were weighed and finally powdered. A portion of powder equivalent to about 1.5 mg SA was weighed accurately and 300 µL dimethyl sulfoxide added, transferred to a 25 mL volumetric flask and stirred with PBS on a magnetic stirrer for 15 min. The solution was filtered and diluted with PBS up to mark. 20 µL volume of sample solution was injected into the column.

Eye drop solution containing 0.1% DIC sodium were used. Total amount of eye drop solution is 5 mL. The solution was filtered and diluted with PBS up to mark. 20 µL volume of sample solution was injected into the column.

RESULTS AND DISCUSSION

The aim of method validation was to confirm that the present method was suitable for its intended purpose as described in International Council for Harmonisation guidelines.[28] The chromatograms of DKP, SA and DIC are given in Figure 1, 2, 3, respectively.

Linearity

The linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of analyte in the sample.[5] Calibration curve of DKP was constructed by plotting absorbance versus concentration which showed linearity over the concentration ranges of 0.39-20 µg/mL (Figure 4). Calibration curve of SA was constructed by plotting absorbance versus concentration which showed linearity over the concentration ranges of 0.0061-0.78 µg/mL (Figure 5). Calibration curve of DIC was constructed by plotting absorbance versus concentration which shows linearity over the concentration ranges of 0.0488-100 µg/mL in Figure 6.

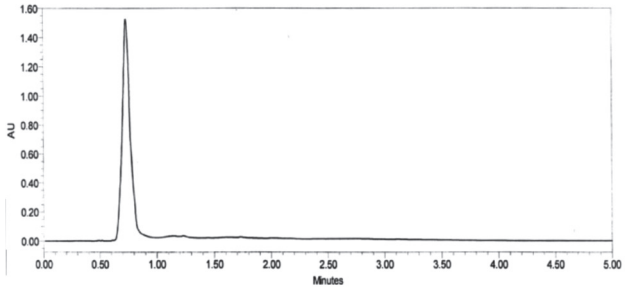


Figure 1. The peak of 1.25 µg/mL concentration of DKP in pH 7.4 phosphate buffer solution, the A solvent was acetonitrile and the B solvent was MeOH/Water (1/1) (v/v), starting conditions were 98% B, and within two min 30% B was employed, the final condition is 98% B, The temperature was 25°C, ultraviolet absorbance data were collected at 254 nm, the flow rate was 0.25 mL/min

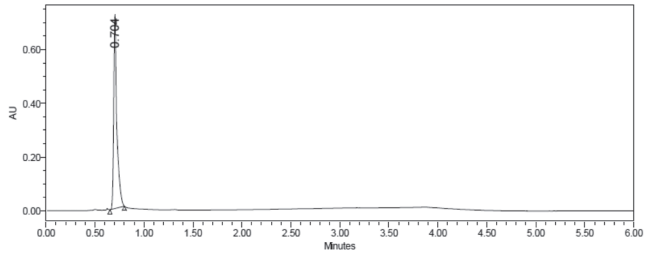


Figure 2. The peak of 1.56 µg/mL concentration of SA in pH 7.4 phosphate buffer solution, the A solvent was acetonitrile and the B solvent was MeOH/Water (1/1) (v/v), starting conditions were 98% B, and within two min 30% B was employed, the final condition is 98% B, the temperature was 25°C, ultraviolet absorbance data were collected at 292 nm, the flow rate was 0.25 mL/min

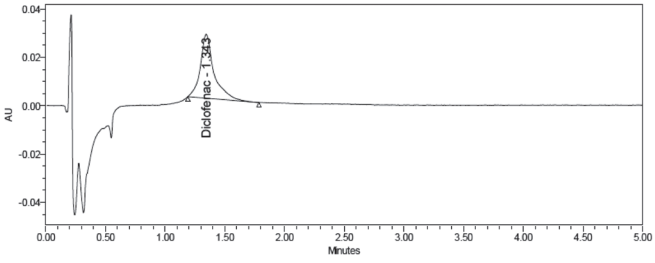


Figure 3. The peak of 3.125 µg/mL concentration of DIC in pH 7.4 phosphate buffer solution, the A solvent was acetonitrile and B solvent was 50 mM acetate buffer (1/1), (v/v) pH 3.1, the column temperature was stable at 25°C, ultraviolet absorbance data were collected at 254 nm

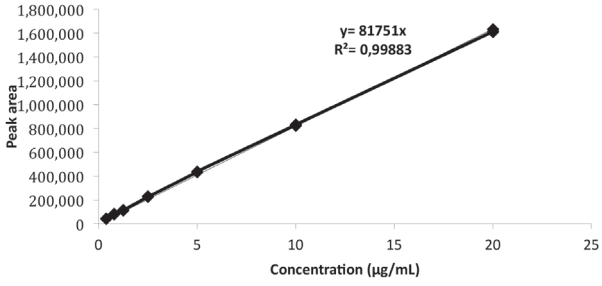


Figure 4. Calibration curve of dexketoprofen trometamol in 25% pH 7.4 phosphate buffer obtained by ultra high performance liquid chromatography method (calibration equations were obtained for a concentration range of 0.39-20 µg/mL)

Accuracy

The accuracy of an analytical method is the closeness of test results obtained by the method to the true value and is defined recovery.[29] As you can see the accuracy results of DKP, SA and DIC in Table 2, 3, 4.

Precision

The precision of an analytical method is the agreement within a series of individual measurements of an analyte when the analytical procedure is applied repeatedly to multiple aliquots of a single homogeneous samples under the same conditions.[19]

Repeatability: In terms of method precision study of our experiment, 15 µg/mL solutions were injected into the system and the percentage of precision was evaluated. Shown in Table 5 shows, the percentage of mean precision value of DKP

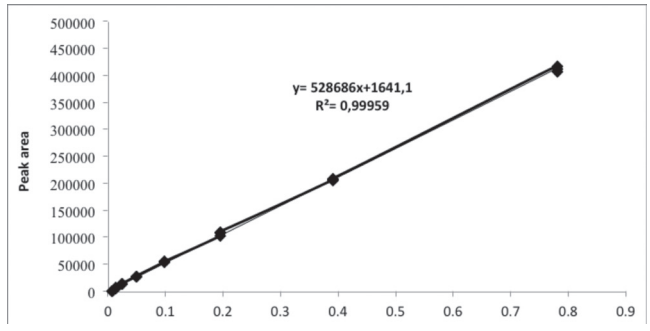


Figure 5. Calibration curve of salicylic acid in 25% pH 7.4 phosphate buffer obtained by ultra high performance liquid chromatography method (calibration equations were obtained for a concentration range of 0.0061-0.78 µg/mL)

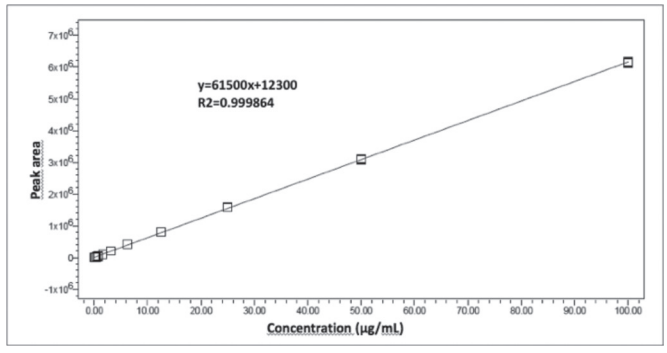


Figure 6. Calibration curve of diclofenac in 25% pH 7.4 phosphate buffer obtained by ultra high performance liquid chromatography method (calibration equations were obtained for a concentration range of 0.0488-100 µg/mL)

Table 2. The calculated recovery amount of DKP from the solution in phosphate buffer

Percentage of DKP in solution	Concentration of DKP in solution (µg/mL)	Recovery of DKP (µg/mL) ± C.I.	Coefficient of variation %
80	2.50	3.01±0.02	0.56
100	3.13	3.85±1.37	1.16
120	3.75	4.56±0.56	0.48

DKP: Dexketoprofen trometamol

concentration level was 108.5 with standard deviation of 1.42. As Table 6 shows, the percentage of mean precision value of SA concentration level was 90.1 with standard deviation of 1.48. As Table 7, 8 shows, the percentage of mean precision value of high DIC concentration and less DIC concentration level were 110, 101 with standard deviation of 1.74, 1.31, respectively. Since the percentage of precision has been found almost 100 and the standard deviation less than the acceptance criteria which is 2%, the analysis system for the determination of assay is verified. Low values of standard deviation denoted very good repeatability of the measurement. Thus it was showing that the equipment used for the study was correct and hence the developed analytical method is highly repetitive.

Reproducibility: To evaluated the reproducibility parameters of DKP stock solution was prepared. Different concentrations of solution were also prepared from the stock solution by dilution. These solutions were measured by UPLC 6 times in 3 consecutive days (Table 9, 10, 11). The obtained to the average of peak heights were calculated standard deviation and standard error of the mean (SEM) values. The average of the concentration, standard deviation and SEM values were calculated. The recovery values from the commercial tablets, test tablets and eye drop, were found to be 103, 114%, 134, 118% and 111, 104% for DKP, SA and DIC respectively. The precision of the chromatographic analysis in tablets and eye drop was determined at two concentrations of each active substance. The coefficients of variation were obtained by repeating the procedure three times for each sample as shown in Table 12, 13, 14.

Table 3. The calculated recovery amount of SA from the solution in phosphate buffer

Percentage of SA in solution	Concentration of SA in solution (µg/mL)	Recovery of SA (µg/mL) ± C.I.	Coefficient of variation %
80	0.31	0.29±1.74	1.96
100	0.39	0.39±0.98	1.03
120	0.47	0.42±1.58	1.84

SA: Salicylic acid

Table 4. The calculated recovery amount of DIC from the solution in phosphate buffer

Percentage of DIC in solution	Concentration of DIC in solution (µg/mL)	Recovery of DIC (µg/mL) ± C.I.	Coefficient of variation %
80	5.00	4.78±1.42	1.85
100	6.25	6.39±1.32	1.78
120	7.50	7.90±1.27	1.46

DIC: Diclofenac

Table 5. The results of precision study for 15.00 µg/mL DKP

Injection number of test solution	Concentration (µg/mL)	Percent value
1	16.60	110.65
2	16.35	109.00
3	16.21	108.09
4	16.08	107.21
5	16.10	107.33
Mean		**108.46**
SD		**1.42**
RSD %		**1.31**

DKP: Dexketoprofen trometamol, RSD: Relative standard deviation, SD: Standard deviation

Specificity/Selectivity

The specificity of an analytical method is its ability to measure accurately and specifically the analyte in the presence of components that may be expected to be present in the sample.[19,30]

Specificity was observed that the diluents did not interfere for the detection of DKP, SA or DIC.

Table 6. The results of precision study for 0.47 µg/mL SA

Injection number of test solution	Concentration (µg/mL)	Percent value
1	0.42	90.06
2	0.41	87.31
3	0.42	90.53
4	0.43	90.95
5	0.43	91.61
6	0.42	90.01
Mean		**90.08**
SD		**1.48**
RSD %		**1.64**

SA: Salicylic acid, RSD: Relative standard deviation, SD: Standard deviation

Table 7. The results of precision study for 15.00 µg/mL DIC (obtained in high DIC concentration)

Injection number of test solution	Concentration (µg/mL)	Percent value
1	16.31	108.72
2	16.41	109.37
3	16.57	110.49
4	16.12	107.47
5	16.83	112.23
6	16.69	111.24
Mean		**109.92**
SD		**1.74**
RSD %		**1.58**

DIC: Diclofenac, RSD: Relative standard deviation, SD: Standard deviation

Table 8. The results of precision study for 3.75 µg/mL DIC (obtained in less DIC concentration)

Injection number of test solution	Concentration (µg/mL)	Percent value
1	3.75	99.88
2	3.76	100.33
3	3.85	102.61
4	3.71	98.95
5	3.81	101.53
6	3.80	101.36
Mean		**100.78**
SD		**1.31**
RSD %		**1.30**

DIC: Diclofenac, RSD: Relative standard deviation, SD: Standard deviation

Table 9. The results of reproducibility of DKP solutions

Concentration (µg/mL)	First day	Second day	Third day	Mean	SD	SEM
1.25	1.55	1.44	1.38	1.46	0.09	0.04
2.50	2.46	2.37	2.32	2.38	0.07	0.03
5.00	5.38	5.28	4.75	5.14	0.34	0.15
10.00	10.49	10.06	9.30	9.95	0.61	0.27
20.00	19.20	21.00	19.50	19.90	0.95	0.43

DKP: Dexketoprofen trometamol, SD: Standard deviation, SEM: Standard error of the mean

Table 10. The results of reproducibility of SA solutions

Concentration (µg/mL)	First day	Second day	Third day	Mean	SD	SEM
0.08	0.07	0.10	0.07	0.08	0.02	0.01
0.16	0.14	0.16	0.14	0.15	0.01	0.00
0.31	0.28	0.26	0.28	0.27	0.01	0.01
0.63	0.54	0.54	0.55	0.54	0.00	0.00

SA: Salicylic acid, SD: Standard deviation, SEM: Standard error of the mean

Table 11. The results of reproducibility of DIC solutions

Concentration (µg/mL)	First day	Second day	Third day	Mean	SD	SEM
2.50	2.50	2.50	2.57	2.53	0.04	0.02
10.00	11.60	11.31	10.52	11.14	0.56	0.25
20.00	23.53	23.04	21.10	22.56	1.28	0.57
40.00	47.16	46.16	42.57	45.30	2.41	1.08
80.00	93.95	91.26	85.89	90.37	4.10	1.84

DIC: Diclofenac, SD: Standard deviation, SEM: Standard error of the mean

The limit of detection and limit of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) tests for the procedure are performed on samples containing very low concentrations of analyses. LOD is defined as the lowest amount of analyze that can be detected above baseline noise; typically, three times the noise level. LOQ is defined as the lowest amount of analyze which can be reproducibly quantitated above the baseline noise.[19,31] LOD was found 0.00325 µg/mL and LOQ was found 0.00985 µg/mL for DKP. LOD was found 0.0027 µg/mL and LOQ was found 0.0081 µg/mL for SA. LOD was found

Table 12. The recovery results of DKP from tablet formulation

The commercial tablet concentration of DKP (µg/mL)	Injection time of test solution	Found concentration of DKP (µg/mL)	% Recovery
1.56	1	1.63	104.49
	2	1.58	101.28
	3	1.59	101.92
	Mean		102.56
	SD		1.70
	RSD %		1.65
12.50	1	14.20	113.60
	2	14.10	112.80
	3	14.30	114.40
	Mean		113.60
	SD		0.80
	RSD %		0.70

DKP: Dexketoprofen trometamol, SD: Standard deviation, RSD: Relative standard deviation

Table 13. The recovery results of SA from tablet formulation

The test tablet concentration of SA (µg/mL)	Injection time of test solution	Found concentration of SA (µg/mL)	% Recovery
0.03	1	0.04	136.52
	2	0.04	133.11
	3	0.04	133.11
	Mean		134.24
	SD		1.97
	RSD %		1.47
0.47	1	0.57	121.60
	2	0.58	123.09
	3	0.52	110.29
	Mean		118.33
	SD		7.00
	RSD %		5.91

SA: Salicylic acid, SD: Standard deviation, RSD: Relative standard deviation

Table 14. The recovery results of DIC sodium from eye drop formulation

The commercial eye drop concentration of DIC (µg/mL)	Injection number of test solution	Found concentration of DIC (µg/mL)	% Recovery
1.56	1	1.75	112.18
	2	1.72	110.26
	3	1.73	110.90
	Mean		111.11
	SD		0.98
	RSD %		0.88
50.00	1	51.50	103.00
	2	52.40	104.80
	3	51.80	103.60
	Mean		103.80
	SD		0.92
	RSD %		0.88

DIC: Diclofenac, SD: Standard deviation, RSD: Relative standard deviation

0.0304 µg/mL and LOQ was found 0.0920 µg/mL for DIC.

LOD= (3.3xσ)/S

LOQ= (10xσ)/S

σ: The standard deviation of the lowest concentration in the calibration range,

S: The slope of the calibration curve (to find the slope of the calibration curve has equation common calibration and taken his slope).

CONCLUSION

A simple, precise, accurate, reproducible, highly sensitive and effective stability indicating UPLC method was developed and validated for quantitative determination of DKP, SA and DIC. The method was validated for accuracy, precision, specificity, and linearity. The developed method has LOD and LOQ values are 0.00325 µg/mL and 0.00985 µg/mL for DKP, respectively. The LOD and LOQ values are 0.0027 µg/mL and 0.0081 µg/mL for SA, respectively. The LOD and LOQ values are 0.0304 µg/mL and 0.0920 µg/mL for DIC, respectively. In this study, the high recovery and low relative standard deviation confirm the suitability of the method for determination of DKP, SA and DIC in pharmaceutical dosage forms. In conclusion, this method can be used for the routine determination of DKP, SA and DIC in pure and pharmaceutical formulations.

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