

New Simultaneous High Performance Liquid Chromatographic Method for Determination of NSAIDs and Opioid Analgesics in Advanced Drug Delivery Systems and Human Plasma

Asad Ullah Madni, Mahmood Ahmad, Naveed Akhtar, Muhammad Usman

Abstract—A new and cost effective RP-HPLC method was developed and validated for simultaneous analysis of non steroidal anti inflammatory drugs Diclofenac sodium (DFS), Flurbiprofen (FLP) and an opioid analgesic Tramadol (TMD) in advanced drug delivery systems (Liposome and Microcapsules), marketed brands and human plasma. Isocratic system was employed for the flow of mobile phase consisting of 10 mM sodium dihydrogen phosphate buffer and acetonitrile in molar ratio of 67: 33 with adjusted pH of 3.2. The stationary phase was hypersil ODS column (C18, 250×4.6 mm i.d., 5 μm) with controlled temperature of 30 C°. DFS in liposomes, microcapsules and marketed drug products was determined in range of 99.76-99.84%. FLP and TMD in microcapsules and brands formulation were 99.78 - 99.94 % and 99.80 - 99.82 %, respectively. Single step liquid-liquid extraction procedure using combination of acetonitrile and trichloroacetic acid (TCA) as protein precipitating agent was employed. The detection limits (at S/N ratio 3) of quality control solutions and plasma samples were 10, 20, and 20 ng/ml for DFS, FLP and TMD, respectively. The Assay was acceptable in linear dynamic range. All other validation parameters were found in limits of FDA and ICH method validation guidelines. The proposed method is sensitive, accurate and precise and could be applicable for routine analysis in pharmaceutical industry as well as in human plasma samples for bioequivalence and pharmacokinetics studies.

Keywords—Diclofenac Sodium, Flurbiprofen, Tramadol, HPLC-UV detection, Validation.

I. INTRODUCTION

PAIN management in current treatment scenario is assumed to be more important than ever before. Combination of NSAIDs and Opioid analgesics (Fig. 1) are being prescribed for the relief pain in different situations of cancers, orthopedics, dental pain, post operative pain management,

osteoarthritis, rheumatoid arthritis and related conditions [1], [2], [3] and [4]. The simultaneous measurement of these NSAID in biological samples is required in therapeutic monitoring, pharmacokinetic and bioavailability studies, as well as in clinical and toxicological screening. Furthermore, it is also very important to precisely quantify these analgesics in advance drug delivery systems and marketed brands for quality control operation [5], [6], [7] and [8].

Diclofenac sodium is an arylpropionic acid derivative belongs to non-steroidal anti-inflammatory drugs (NSAIDs), which have analgesics, antipyretics and anti-inflammatory activities. Mechanism of action is related to inhibition of cyclooxygenase (COX) and reduces prostaglandin biosynthesis at the site of inflammation [9]. Flurbiprofen is a propionic acid-derived non-steroidal anti-inflammatory drug (NSAID) used widely in the treatment of rheumatism and non-arthritic pain and its use is also approved in fever and platelet aggregation [10]. Tramadol hydrochloride (trans-2-[(dimethyl-amino) methyl] -1-(methoxypheny) cyclohexanol, centrally acting opioid agonist provide analgesic effect by blocking μ-receptors [11].

Multiple separation and analysis techniques have been developed for the simultaneous and single entity determination of different NSAIDs in formulations and biological fluids. But no method is available for simultaneous determination of DFS, FLP and TMD in drug products and biological fluids. The available methods for different combination of analgesics mainly employed spectroscopic and micro colorimetric assay [12], [13], [14], and [15], spectrofluorimetry [16], HPLC with crocheted ETEF post column photo derivatization [17], HPLC with ultraviolet [6], [8], [9], [10], [11], [18], [19], [20] electrochemical detection (ECD) [7], HP-TLC method [21], and potentiometric analysis [22], thermal and raman spectroscopic method [23], and [24]. Spectrophotometric, fluorometric, potentiometric, thin layer chromatographic method and thermal and raman methods are slow and difficult methods lacking sensitivity and selectivity required for multidrug analysis in different fluids, HPLC analysis being fast, sensitive, specific and selective for multidrug analysis replacing these methods. While mass spectrometric (MS) with different assemblies are being costly and cannot be employed for routine analysis of drugs in formulations and biological samples. Elessia P et al. (2007)

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used UV and ESI-MS detection employing liquid chromatography for eight different drugs in formulations, although the technique is very sensitive but peaks of ketoprofen and naproxen interfered with each other. The technique is not available in most of the laboratories therefore, making the method expensive and cannot be employed in biological fluid analysis having large number of samples. Yen Sun et al, (2003) presented HPLC method for determining seven kinds of NSAIDs simultaneously in pharmaceutical formulations and human plasma. Although the method was simple but separation was compromised, as naproxen and fenoprofen were not sufficiently separated from endogenous peaks and the chromatograph was not clear [20]. Compared to method developed by Toshio H. et al, 1997; employing SPE for determination of twelve drugs that was quite time consuming, expensive, inappropriate separation of drugs shown in chromatograms and requires large volumes of solvents for sample preparation resulted in less sensitivity compared to present method [8]. In this study, we have employed variable UV detector with no special assembly which proved to be convenient, inexpensive and suitable for routine work, an isocratic separation of analgesic drugs only using reversed phase column was also found better for the separation of these drugs. In spite of analyzing more drugs, it is better to analyze and separate less number of drugs to avoid the problem of interference and peak symmetry. The proposed method was successfully applied for the determination of each drug simultaneously in drug delivery systems, commercial brands as well as in human plasma samples.

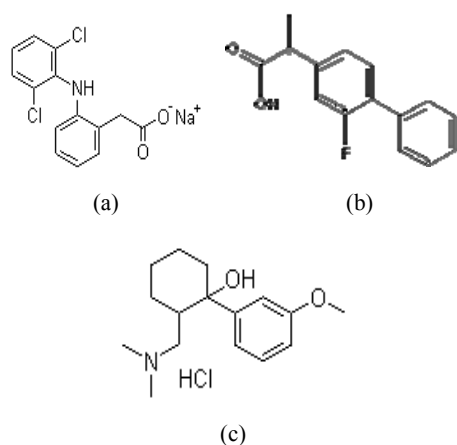


Fig. 1 Structure of (a) DFS (b) FLP (c) TMD

II. EXPERIMENTAL

A. Materials

Diclofenac sodium (DFS), Flurbiprofen (FLP) and Tramadol hydrochloride (TMD) were donated by Novartis Pharmaceutical (Pakistan), Pfizer Pharmaceutical (Pakistan) Ltd. and Getz Pharmaceuticals (Pakistan) Ltd, respectively. Analytical grade Sodium dihydrogen phosphate, phosphoric acid, methanol, acetonitrile and trichloroacetic acid were purchased by Merck (Darmstadt, Germany). Double distilled water was prepared in laboratory (Pharmacy Department, the Islamia University of Bahawalpur-Pakistan).

B. Chromatographic System and Applied Conditions

The Sykam GmbH (Germany) HPLC system consisted of S-2100 solvent delivery system (Germany), VE 3210 variable wavelength UV/VIS detector, Rheodyne (Sykam GmbH, Germany) sample injector with a 20- μ L loop. Clarity DataApex[®] software (Sykam GmbH, Germany) was used as interface modulator for data processing. Chromatographic separations were carried out on a hypersil ODS column (250 mm \times 4.6 mm i. d., 5 μ m particle size of internal packing, Germany) preceded by guard column. S 4011 Column Thermo Controller (Sykam GmbH, Germany) was used to control and maintain column temperature at 30 C[°]. Isocratic separations were carried out with optimized composition of mobile phase. After several trials, a mobile phase of composition acetonitrile-sodium dihydrogen phosphate buffer (pH 3.5; 10m M)-acetonitrile (33:67, v/v) with an optimized flow rate of 1.0 ml.min⁻¹. The effluent was monitored at 242 nm for 0–5min, 274 nm 5–11 minutes.

C. Preparation of Standard Solutions

100 μ g.ml⁻¹ standard stock solutions of DFS, FLB and TMD were prepared by dissolving 100 mg of each drug in 100 ml of acetonitrile. These stock solutions were further diluted to make working dilutions to encompass linearity range of each drug. Standard dilutions were analyzed in HPLC. Standard dilutions of DFS were made in the range of 10 ng.ml⁻¹ to 20 μ g.ml⁻¹, FLB and TMD were made in standard dilutions of 20 ng.ml⁻¹ to 10 μ g.ml⁻¹, respectively. Same dilutions were prepared in human plasma after spiking known drug solution concentrations with plasma. Liquid-Liquid Extraction procedure was adopted for sample preparation HPLC system.

D. Determination of Analgesics in Marketed Brands

Ten tablets of DFS, FLB and TMD and 10 capsules of DFS and FLP in sustained release marketed brands were finely ground. An accurately weighed powdered sample containing the labeled amount of each drug was transferred to a 100 ml volumetric flask. The volume was adjusted with acetonitrile and the resultant solution was sonicated for 5 min. A portion of the solution was then filtered through a 0.45 μ m filter. 1ml of filtrate was diluted to 50 ml and a portion of 20 μ L was injected to HPLC system.

E. Determination of Analgesics in Drug Delivery Systems

Liposomes were prepared in laboratory by modified microencapsulation vesicle method by Tomokio N. and Fumiyoshi I., 2005 [25]. Microcapsules of DFS, TMD and FLP were prepared by coacervation based on temperature change technique used by Sajeev C. et al., 2004 [26]. The formed liposomes and microcapsules equivalent to one dose were weighed and dissolving in acetonitrile and vortex mixed, centrifuged, and filtered through 0.45 μ m filter and 1 ml of filtrate was diluted to 50 ml and injected through 20 μ L loop into HPLC system.

F. Determination of NSAIDs in Human Plasma

Blood samples were withdrawn and centrifuged to separate plasma and kept in ultra low freezer (-20 °C) until analysis performed. LLE was performed by taking 250 µL of human plasma in glass tube and 200 µL precipitant was added and vortex mixed for 1 minute and centrifuged for 5 minutes. The clear supernatant was separated and injected into HPLC column for separation.

III. RESULTS AND DISCUSSION

A. Choice of Chromatographic Separations

The isocratic elution system was found easier and economical for separation of analgesics in formulations and plasma samples using an optimized composition of mobile phase, mixture of acetonitrile-10 mM sodium dihydrogen phosphate buffer (pH 3.2). The value of retention factor (k') of each drug was reduced as the value of pH of mobile phase above 3.5, while diminutive changes were found up to pH 3.5; the pH 3.2 was chosen as the best condition. The proposed separation condition was applied to the analysis of NSAIDs in pharmaceutical formulations. Though, TMD interfered with the with modifier retention and peak of plasma by using methanol which was selected at initial stages but with adjustment of pH of buffer to pH 3.2 and slight adjustment of composition in mobile phase (33:67 acetonitrile and phosphate buffer) a complete separation of study entities in plasma was made possible (Fig. 2). Each drug contains different chromophores (light absorbing units) for variation in maximum wavelength of ultraviolet absorbance. Variable wavelength detector was set at different λ_{max} i.e. 242 nm for 0-5min, 274 nm 5-11 min.

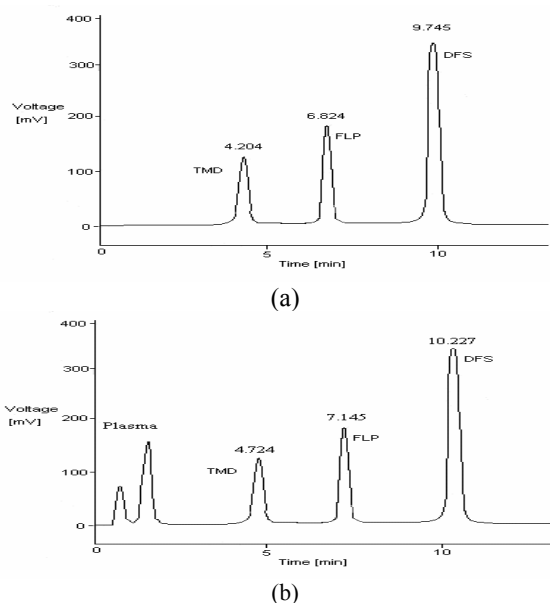


Fig. 2 Chromatograms showing (a) separation of analgesics in drug solution (b) plasma samples

B. Determination of Analgesics in Pharmaceutical Formulations

The Accuracy is a measure of deviation of mean from the true value as determined by the replicate assay. The accuracy of the present method was found in the range of 99.76 to 99.99 for drug solution and 99.65- 99.88 for drug solutions samples for the drug concentrations of LQC, MQC and HQC (Table I). The measure of the amount of agreement among the observed results when method is applied repeatedly and reproducibly is called precision. The precision (% C.V) for all the studied components in different fluids were found in the recommended limits and summarized in Table II. The results of analysis of liposomes, microcapsules and marketed brands, are shown in Table III.

TABLE I
 WITH-IN-DAY ACCURACY AND PRECISION OF ANALGESICS IN DRUG SOLUTIONS

Curve Code	LQC (ng.ml ⁻¹)			MQC (µg.ml ⁻¹)			HQC (µg.ml ⁻¹)		
	DFS	FLP	TMD	DFS	FLP	TMD	DFS	FLP	TMD
Batch 01	9.86	19.94	19.28	0.995	0.998	0.992	9.98	19.98	19.92
Batch 02	9.91	19.98	19.34	0.992	0.986	0.994	9.94	19.96	19.84
Batch 03	9.88	19.92	19.81	0.998	0.979	0.996	9.881	19.96	19.9
Batch 04	9.84	19.96	18.96	0.969	0.996	0.995	9.96	19.94	19.96
Batch 05	9.87	19.98	18.81	0.996	0.998	0.988	9.61	19.97	19.81
Batch 06	9.85	19.95	19.72	0.994	0.994	0.996	9.92	19.98	19.88
Mean	9.87	19.96	19.32	0.99	0.99	0.99	9.88	19.97	19.89
S.D.	0.02	0.02	0.40	0.01	0.01	0.00	0.14	0.02	0.05
Nominal	10	20	20	1	1	1	10	20	20
%CV	0.25	0.12	2.06	1.09	0.78	0.31	1.39	0.08	0.27
%Bias	-1.32	-0.22	-3.40	-0.93	-0.82	-0.65	-1.18	-0.18	-0.57
%Accuracy	98.68	99.78	96.60	99.07	99.18	99.35	98.82	99.83	99.43

TABLE II
 WITH-IN-DAY ACCURACY AND PRECISION OF ANALGESICS IN DRUG SOLUTIONS

Curve Code	DFS			FLP			TMD		
	LQC	MQC	HQC	LQC	MQC	HQC	LQC	MQC	HQC
Units	ng. ml ⁻¹	µg. ml ⁻¹	µg. ml ⁻¹	ng. ml ⁻¹	µg. ml ⁻¹	µg. ml ⁻¹	ng. ml ⁻¹	µg. ml ⁻¹	µg. ml ⁻¹
Day-01	9.98	19.99	19.96	0.996	2.49	2.48	9.96	19.98	19.98
	9.99	19.95	19.94	0.995	2.49	2.49	9.98	19.96	19.96
	9.99	19.98	19.97	0.998	2.48	2.49	9.92	19.97	19.97
Day-02	9.98	19.97	19.96	0.992	2.48	2.48	9.96	19.98	19.94
	9.95	19.96	19.98	0.991	2.49	2.47	9.94	19.92	19.95
	9.95	19.98	19.94	0.996	2.48	2.48	9.98	19.96	19.94
Day-03	9.97	19.96	19.94	0.990	2.49	2.49	9.92	19.98	19.96
	9.96	19.94	19.94	0.988	2.48	2.48	9.96	19.94	19.97
	9.95	19.94	19.92	0.992	2.49	2.48	9.95	19.96	19.98
Mean	9.97	19.96	19.95	0.99	2.49	2.48	9.95	19.96	19.96
S.D.	0.017	0.018	0.02	0.003	0.005	0.07	0.02	0.02	0.02
Nominal	10	20	20	1	2.5	2.5	10	20	20
%CV	0.17	0.09	0.09	0.33	0.21	0.27	0.22	0.10	0.08
%Bias	-0.31	-0.18	-0.25	-0.69	-0.58	-0.71	-0.48	-0.2	-0.19
%Accuracy	99.69	99.82	99.75	99.31	99.42	99.29	99.52	99.81	99.81

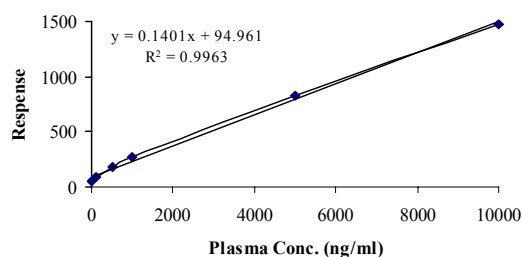
TABLE III
 PERCENTAGE CONTENTS FOUND IN LIPOSOMES, MICROCAPSULES AND MARKETED BRANDS

Formulations	Drug/Brands	Stated	Found	Percentage
Liposome	DFS	50	49.86	99.72
	DFS	50	49.93	99.86
Microcapsule	FLP	100	99.95	99.95
	TMD	100	99.72	99.72
Marketed brands	Dicloran [®] Sami	50	49.88	99.76
	Voltral [®] Novartis	50	49.92	99.84
	Froben [®] Abbott	100	99.94	99.94
	Ansaid [®] Pfizer	100	99.78	99.78
	Tramal [®] Searle	50	49.9	99.8
	Traumanil [®] Howards	50	49.91	99.82

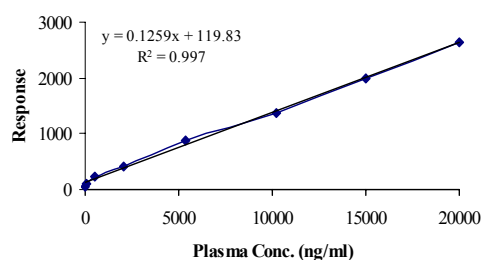
C. Determination of Analgesics in Human Plasma

1. Linearity

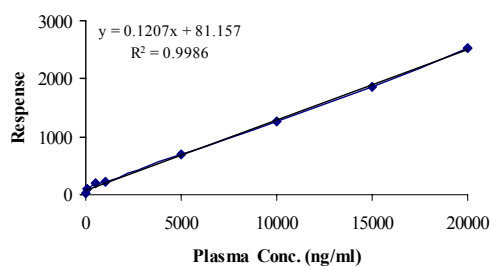
Three replicates of each concentration in the dynamic range of 0.01–10 µg⁻¹ for DFS and 0.01-20 µg.ml⁻¹ for TMD and FLP were run in plasma matrix. A linear relationship (Fig. 3) was obtained between the peak area and concentration for each drug (Table IV). Limit of quantifications (LOQs) of each compound was first concentration of each drug which was set at three fold of limits of detections (LODs) at a signal-to-noise ratio of 3. The linearity of the present method was comparable to the methods [5], [6] and better when compared with those of other publications [7], [8], [9].



(a)



(b)



(c)

Fig. 3 Standard curve of (a) DFS (b) FLP and (c) TMD in plasma

TABLE IV
STANDARD CURVE PARAMETERS OF ANALGESICS IN PLASMA

Curve Code	Slope Mean (%C.V)	Intercept Mean (%C.V)	r-square Mean (%C.V)
DFS	94.96 (0.14)	0.14 (0.84)	0.996 (0.05)
FLP	119.83 (0.62)	0.125 (1.36)	0.998 (0.08)
TMD	70.8 (1.26)	78.6 (0.48)	0.995 (0.44)

2. Extraction efficiency

Efficiency of the extraction method was determined for each drug at lowest concentration and highest concentration in plasma by comparing the peak heights of each compound spiked in plasma, to those of standards in the mobile phase. Several trials were done to find out adequate extraction solvent for withdrawal of drugs from plasma matrix to form easier method of liquid-liquid extraction and TCA with acetonitrile was found satisfactory. The recoveries for analgesics were 99.03–99.80 at lowest, middle and highest concentrations of standard curve (Table V). These findings were higher than studies available in literature [9], [10], [12], [13], [19].

TABLE V
EXTRACTION EFFICIENCY OF ANALGESICS IN PLASMA

Code	LQC (Mean, %C. V.)			HQC (Mean, %C. V.)		
	Ext.	Non-Ext.	%	Ext.	Non-Ext.	%
DFS	9.70 (1.73)	9.75 (1.72)	99.52	9.91 (0.15)	9.95 (0.31)	99.60
FLP	9.77 (0.43)	9.80 (0.42)	99.69	19.91 (0.08)	19.95 (0.15)	99.80
TMD	9.84 (0.82)	9.93 (0.81)	99.03	19.85 (0.10)	19.92 (0.18)	99.63

3. Assay accuracy and precision in plasma

Intra-day accuracy and precision of the assay was performed six batches spikes plasma samples with DFS at low (0.01 µg/ml), middle (1.0 µg/ml) and high (10 µg/ml) concentrations, FLP and TMD were spiked at low (0.02 µg/ml), middle (1.0 µg/ml) and high (20 µg/ml) concentrations. Triplicate samples at each concentration were analyzed for the intra-day assessment and finding system error (biasness). For the inter-day precision (measure of repeatability and reproducibility) analysis of the spiked plasma at the same concentrations of each drug was performed on 3 different days. The results are given in Table VI and Table VII.

TABLE VI
WITH-IN-DAY ACCURACY AND PRECISION IN PLASMA

Curve code	LQC (ng.ml-1)			MQC (µg.ml-1)			HQC (µg.ml-1)		
	DFS	FLP	TMD	DFS	FLP	TMD	DFS	FLP	TMD
Batch 01	9.82	19.28	19.28	0.995	0.998	0.992	9.98	19.88	19.92
	9.88	19.34	19.34	0.992	0.986	0.994	9.94	19.96	19.84
	9.86	19.81	19.81	0.998	0.979	0.996	9.881	19.96	19.9
	9.85	18.96	18.96	0.969	0.996	0.995	9.96	19.84	19.96
	9.90	18.61	18.81	0.996	0.998	0.988	9.61	19.87	19.81
	9.82	19.72	19.72	0.994	0.994	0.996	9.92	19.92	19.88
Mean	9.86	19.29	19.32	0.99	0.99	0.99	9.88	19.91	19.89
S.D.	0.03	0.45	0.40	0.01	0.01	0.00	0.14	0.05	0.05
Nominal	10	20	20	1	1	1	10	20	20
%CV	0.31	2.35	2.06	1.09	0.78	0.31	1.39	0.25	0.27
%Bias	-1.38	-3.57	-3.40	-0.93	-0.82	-0.65	-1.18	-0.48	-0.58
%Acc	98.62	96.43	96.60	99.07	99.18	99.35	98.82	99.53	99.43

TABLE VII
BETWEEN-THE-DAY ACCURACY AND PRECISION IN PLASMA

Curve Code	DFS			FLP			TMD		
	LQC	MQC	HQC	LQC	MQC	HQC	LQC	MQC	HQC
Units	ng/ml	µg/ml	µg/ml	ng/ml	µg/ml	µg/ml	ng/ml	µg/ml	µg/ml
Batch-01	9.96	19.98	19.94	0.996	2.48	2.47	9.86	19.96	19.95
	9.94	19.94	19.92	0.995	2.49	2.48	9.95	19.94	19.93
	9.98	19.96	19.95	0.998	2.47	2.49	9.94	19.92	19.94
Batch-02	9.93	19.94	19.90	0.992	2.46	2.48	9.92	19.88	19.92
	9.90	19.92	19.88	0.991	2.47	2.46	9.92	19.94	19.90
	9.92	19.91	19.84	0.996	2.46	2.48	9.94	19.94	19.92
Batch-03	9.95	19.92	19.89	0.990	2.49	2.47	9.22	19.92	19.90
	9.94	19.92	19.82	0.988	2.48	2.48	9.86	19.92	19.94
	9.92	19.90	19.80	0.992	2.49	2.47	9.89	19.94	19.88
Mean	9.94	19.93	19.88	0.99	2.487	2.476	9.83	19.93	19.92
S.D.	0.02	0.025	0.053	0.003	0.012	0.008	0.23	0.023	0.023
Nominal	10	20	20	1	2.5	2.5	10	20	20
%CV	0.24	0.13	0.26	0.33	0.49	0.36	2.36	0.11	0.12
%Bias	-0.62	-0.34	-0.59	-0.69	-0.93	-0.98	-1.67	-0.4	-0.40
%Acc	99.38	99.66	99.41	99.31	99.07	99.02	98.33	99.64	99.60

4. Stability

The stability analgesic drug was studied in acetonitrile on four weeks to observe change in composition of solution. These studies were conducted at lowest and highest concentrations (extremes) of each drug. The value of (%CV) for DFS was 0.35 for LQC and 0.09 for HQC was observed; similarly it was 0.13 and 0.15 for LQC and 0.22 & 0.25 HQC for FLP and TMD, respectively (Table VIII). The freeze-thaw stability of analgesics was also approximated over three freeze-thaw cycles in plasma. Lowest and highest concentrations of each drug were spiked in plasma, frozen at -25 °C and thawed at room temperature for three successive events. Table IX furnishes freeze-thaw stabilities of DFS and found %CV (LQC, -1.80 to -2.01; HQC, -0.34 to -0.54). The values for FLP and TMD were summarized in Table X and Table XI, respectively. These findings specify that analgesics were stable in processing for finding concentration after freezing for holding the samples in ultra low freezer. These values were also found in good agreement with previous studies and indicate that analgesics in drug solutions are stable for one month and spiked plasma after three freeze-thaw cycles.

TABLE VIII
LONG TERM STABILITY OF ANALGESICS IN DRUG SOLUTIONS

Dilutions	Week1	Week2	Week3	Week4	Mean	S.D.	%CV
DFS LQC	9.94	9.91	9.88	9.86	9.89	0.03	0.35
DFS HQC	19.97	19.95	19.94	19.93	19.95	0.02	0.09
FLP LQC	19.96	19.94	19.92	19.9	19.93	0.03	0.13
FLP HQC	19.98	19.94	19.90	19.88	19.93	0.04	0.22
TMD LQC	19.95	19.92	19.9	19.88	19.91	0.03	0.15
TMD HQC	19.98	19.92	19.94	19.86	19.93	0.05	0.25

LQC=Lowest quality control concentration in ng/ml,, HQC= Highest quality control concentration in µg/ml

TABLE IX
FREEZE-THAW STABILITY OF DFS IN PLASMA

Curve Code	Cycle							
	Cycle 0		Cycle 1		Cycle 2		Cycle 3	
	LQC	HQC	LQC	HQC	LQC	HQC	LQC	HQC
DFS 01	9.68	9.56	9.52	9.81	9.54	9.75	9.46	9.97
	9.81	9.98	9.91	9.71	9.42	9.68	9.81	9.64
	9.89	9.92	9.42	9.84	9.83	9.79	9.59	9.69
Mean	9.79	9.82	9.62	9.79	9.60	9.74	9.62	9.77
S.D.	0.11	0.23	0.26	0.07	0.21	0.06	0.18	0.18
Nominal	10	20	10	20	10	20	10	20
%CV	1.08	2.31	2.69	0.69	2.20	0.57	1.84	1.82
%Diff	-	-	-1.80	-0.34	-2.01	-0.81	-1.77	-0.54

LQC=Lowest quality control concentration in ng/ml,
HQC= Highest quality control concentration in µg/ml

TABLE X
FREEZE-THAW STABILITY OF FLP IN PLASMA

Curve Code	Cycle							
	Cycle 0		Cycle 1		Cycle 2		Cycle 3	
	LQC	HQC	LQC	HQC	LQC	HQC	LQC	HQC
FLP 01	19.96	19.86	19.87	19.78	19.45	19.68	18.85	19.75
	19.12	19.79	18.78	19.85	19.52	19.81	19.75	19.62
	19.97	19.84	19.1	19.71	19.7	19.10	18.62	19.45
Mean	19.68	19.83	19.25	19.78	19.56	19.53	19.07	19.61
S.D.	0.4879	0.0361	0.5603	0.0700	0.1290	0.3780	0.5972	0.1504
Nominal	20	20	20	20	20	20	20	20
%CV	2.48	0.18	2.91	0.35	0.66	1.94	3.13	0.77
%Diff	-	-	-2.20	-0.25	-0.64	-1.51	-3.10	-1.13

TABLE XI
FREEZE-THAW STABILITY OF TMD IN PLASMA

Curve Code	Cycle							
	Cycle 0		Cycle 1		Cycle 2		Cycle 3	
	LQC	HQC	LQC	HQC	LQC	HQC	LQC	HQC
TMD 01	19.86	19.86	19.78	19.77	19.55	19.72	19.42	19.7
	19.92	19.95	18.88	19.87	19.68	19.83	19.60	19.72
	19.92	19.94	19.85	19.85	19.77	19.75	19.62	19.65
Mean	19.90	19.92	19.50	19.83	19.67	19.77	19.55	19.69
S.D.	0.03	0.05	0.54	0.05	0.11	0.06	0.11	0.04
Nominal	20	20	20	20	2	20	20	20
%CV	0.17	0.25	2.77	0.27	0.56	0.29	0.56	0.18
%Diff	-	-	-1.99	-0.44	-1.17	-0.75	-1.78	-1.14

IV. CONCLUSION

A simple and inexpensive simultaneous high performance liquid chromatographic method for the determination of analgesics employing UV detection was developed and found successful in analysis of marketed brands, drug delivery systems and human plasma samples. The validation parameters were also found in acceptable limits of FDA and ICH. LOQs of each drug in plasma were as low as 10 ng/ml for DFS and 20 ng/ml for FLP and TMD which was enough for monitoring the blood levels of each entity. The method was found accurate, precise and stable that makes it applicable for quality control operations in pharmaceutical industry, pharmacokinetics studies and therapeutic drug monitoring of these drugs in clinical settings.

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