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Spectrofluorometric determination of drugs containing α-methylene carbonyl functional group using N^1-methylnicotinamide chloride as a fluorogenic agent

Rofaida A.Salem, Mohamed A.Eldawy, Mohamed A.Elkersh, Mervat H.El-Hamamsy*
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Tanta University, Tanta 31527, (EGYPT)
E-mail: mhamamsy_7@yahoo.com

ABSTRACT

A simple spectrofluorometric method has been developed, adapted, and validated for analysis of certain drugs containing active methylene group adjacent to carbonyl functional group. This method was applied successfully for quantitative determination of drugs having cyclic α-methylene carbonyl groups in a cyclohexanone, cycloheptanone and macromolecular structures such as Naltrexone (I), Ketotifen (II), Oxcarbazepine (III) and Pimecrolimus (IV) respectively in various pharmaceutical dosage forms. The fluorescence of the products resulting from the reaction between tested drugs and N^1-methylnicotinamide chloride (NMNCl) have been measured in pure pharmaceutical ingredient, laboratory-prepared mixtures, pharmaceutical dosage forms and spiked human plasma samples. The developed method is in line with all current compendial criteria. The proposed method is simple, with low instrumentation requirements, suitable for quality control application, bioavailability and bioequivalence studies.

KEYWORDS

Spectrofluorometry;
Naltrexone;
Ketotifen;
Oxcarbazepine;
Pimecrolimus;
N^1-methylnicotinamide chloride.

INTRODUCTION

It was reported that compounds having α-methylene next to carbonyl group (R-CH_2-CO-R‘) produce fluorescent product upon reaction with NMNCl[1-3]. The subject matter of this manuscript builds upon the successful adaptation in our laboratories of the reaction between NMNCl and (R-CH_2-CO-R‘) containing compounds[4,5]. This adaptation involved the extension of this reaction to drugs that contain the active α-methylene carbonyl group in open chain structures[4,5]. Further; the methodology was also adapted for the analysis of compounds containing endocyclic α-methylene carbonyl functional group (-CH_2-CO-)[6]. The methodology was also successfully applied for determination of drugs containing a similar to α–methylene carbonyl functional group such as; drugs containing an α– methylene sulfoxide[7] moiety in addition drugs containing an α–methylene sulfone/sulfonamide functional groups[8]. The reaction of this reagent with in situ generated α–methylene carbonyl group which is produced from a number of synthetic steroidal derivatives containing a terminal methyne functional group was also developed[9]. It seemed interesting to investigate the possibility of adapting this methodology to determi-

nation of opoid antagonists containing endocyclic six membered α-methylene carbonyl group included in rigid structures such as Naltrexone (I). Naltrexone (I) was chosen to investigate the effect of rigidity on the generation of the fluorophore in view of Nakamura's findings that rigid compounds failed to react[3].

AThis method was applied for quantitative determination of drugs containing cyclic α–methylene carbonyl groups included in cycloheptanone ring structure like Ketotifen (II) and Oxcarbazepine (III). Also we investigated the practicalities of generating a fluorophore for α-methylene carbonyl group in macrocyclic compound, Pimecrolimus (IV) Figure (1).

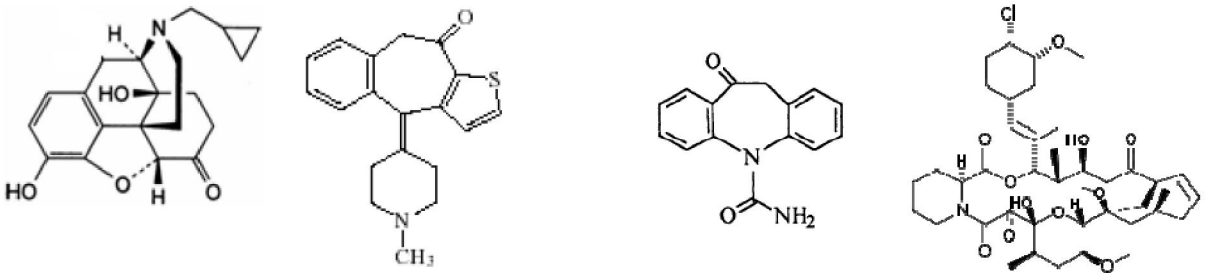
Naltrexone (I) is a long-acting synthetic competitive narcotic antagonist at the opioid mu-receptor that is important in the detoxification of opiate addicts[10].

Naltrexone is available as 50 mg tablets, 380-mg extended release injection (vivitrol ®)[11,12] and investigational implants[13,14]. Naltrexone has been determined by using a wide variety of analytical techniques, including combination of chromatographic methods with electrochemical[15], mass[16,17], tandem mass[18] and amperometric[19] detection. Chemiluminescence[20] has also been used for the determination of Naltrexone.

Ketotifen (II) has antiallergic properties and a selective H1 antagonist effect. The drug is available as 1mg tablets and 1mg/5ml oral solution[21]. Different chromatographic methods were reported for analysis of Ketotifen with electrochemical[22], mass[23, 24], tandem mass[25] detection. Ketotifen also determined by atomic absorption[26] and colorimetric methods[27]. Coulometric titration of Ketotifen fumarate is also a method reported for its determination[28]. Moreover; it was determined using differential pulse polarography[29] and iontransfer voltammetry[30]. Chemiluminescence[31] has also been used for determination of Ketotifen.

Oxcarbazepine (III) an antiepileptic drug available as 150 mg, 300 mg and 600 mg film-coated tablets. Oxcarbazepine is also available as a 300 mg/5 ml (60 mg/ml) oral suspension. A number of chromatographic methods for the quantification of Oxcarbazepine and its main metabolites in biological fluids are already published however; some of these methods are not sufficiently sensitive and time-consuming[32]. Combinations of chromatographic methods with electrospray tandem mass[33] and mass detection in positive chemical ionization mode[34] seem to be the most important methods for determination of (III). Electrochemical techniques provide an interesting alternative to the chromatographic methods such as Square Wave Adsorptive Stripping Voltammetry[35].

Pimecrolimus (IV); an immunomodulating agent used in treatment of atopic dermatitis (eczema)[36]. A new study of Pimecrolimus-Eluting (325μg) (Corio™) and dual drug Pimecrolimus and Paclitaxel eluting (162.5μg/10μg) (SymBio™) coronary stent systems in patients with de novo lesions of the native coronary arteries suspended in 2007[37]. Different methods have been reported for immunosuppressant determination including several LC methods coupled with mass spectrometry or tandem mass spectrometry (MS/MS) detection[38]. Several immunological methods are developed for whole blood quantification of immunosuppressant drugs including enzyme multiplied immunoassay technique (EMIT)[39] and enzyme-linked immunosorbent assay



(I) (II) (III) (IV)

Figure 1 : Chemical structures of Naltrexone (I) Ketotifen (II), Oxcarbazepine (III) and Pimecrolimus (IV)

(ELISA)[40].

Our newly developed method is simple compared to other reported methodologies that require elaborate, expensive instrumentation and time-consuming sample preparation procedures in addition to the built in high sensitivity of the fluorometric technique.

EXPERIMENTAL

Apparatus.

Fluorescence was detected using Shimadzu RF 5301 PC spectrofluorometer.

Materials

Authentic drug

The working standard of (I) was supplied by Deltapharma Company. The working standard of (II) was supplied by Novartis Pharma S.A.E., Memphis Pharmaceutical and Chemical Industries Company and Pharco Pharmaceuticals (Egypt). The working standard of (III) and (IV) were supplied by Novartis Pharma S.A.E. Plasma samples were purchased from the Central Blood Bank of Tanta University Hospital

Chemicals

N^1-Methylnicotinamide chloride was purchased from Sigma Chemicals Co. Formic acid, sodium hydroxide, methanol, and all other chemicals were of analytical grade. Water used was doubly distilled.

Dosage forms

Naltrexone (I): Deltrexone 50 mg tablets (Delta pharma.).

Ketotifen (II): Zedotefen1mg tablets, 1 mg/5 ml syrup and 0.025% eye drops (Nasr Pharmaceutical & Chemicals Industries Company), Zaditen1mg tablets, 1 mg/5 ml syrup and 0.025% eye drops (Novartis Pharma S.A.E.), Zylofen1mg tablets and 1 mg/5 ml syrup (Memphis Pharmaceutical & Chemicals Industries Company) and Ketoti 1mg tablets and 1 mg/5 ml syrup (Pharco. Pharma-ceuticals).

Oxcarbazepine (III); Trileptal 150, 300 and 600 mg tablets (Novartis Pharma S.A.E.).

Pimecrolimus (IV): Elidel 1% cream (Novartis Pharma S.A.E.)

Reagents and standard solutions

Stock standard solutions of drugs

Stock solution for (I) was prepared in water to contain 0.5 mg/ml while stock solutions for (II), (III) and (IV) were prepared in methanol to contain 0.1 mg/ml, 0.5 mg/ml and 0.1 mg/ml respectively.

Serial standard solutions of drugs

Aliquots of the stock solutions were diluted quantitatively with the same solvent to obtain serial standard solutions in concentration ranging from 2 to 50 ng/ml, 0.1 to 20 ng/ml, 0.5 to 60 μg/ml and 0.2 to 20 ng/ml of I, II, III and IV respectively.

Assay solutions of drugs in synthetic mixtures

Synthetic mixture containing (I) along with various excipients, additives and other non active ingredients commonly used in pharmaceutical formulations were prepared.

The mixture contained (50 mg) of Naltrexone, (8.89 mg) crospovidone, (5.08 mg) hydroxypropyl methylcellulose, (2.54 mg) magnesium stearate, (7.62 mg) polyethylene glycol, (7.62 mg) polysorbate 80, (1.27 mg) silicon dioxide, (2.54 mg) titanium dioxide, (2.54 mg) yellow iron oxide and (2.54 mg) red iron oxide, (2.54 mg) lactose monohydrate, and microcrystalline cellulose to 254 mg.

Three synthetic mixture containing (II) were prepared. The first mixture contained (1.30 mg) of Ketotifen fumarate, (4.90 mg) magnesium stearate, (9.85 mg) ac.di.sol and avicel pH 101 to (98.50 mg). The second mixture contained (0.27 g) of Ketotifen fumarate, (0.10 g) flavor, (0.17 g) propyl paraben, (0.33 g) methyl paraben, (2.10 g) citric acid anhydrous, (3.20 g) disodim hydrogen phosphate anhydrous, (20 g) ethanol, (300 g) sucrose, (350 g) sorbitol and q.s water purified to 1 liter. The third mixture contained (1.25 mg) Ketotifen fumarate, (0.364 g) benzalkonium chloride, (0.274 g) glycerin and purified water to complete 5 ml.

One synthetic mixture containing (III) were prepared. The mixture contained (150 mg) of Oxcarbazepine, (3.75 mg) colloidal silicon dioxide, (22.5 mg) crospovidone, (26.25 mg) hydroxypropyl

methylcellulose, (7.5 g) magnesium stearate, (30 mg) polyethylene glycol, (7.5 mg) talc, (7.5 mg) titanium dioxide, (7.5 mg) yellow iron oxide and microcrystalline cellulose to 750 mg.

One mixture contained 1g of (IV), The mixture contained (0.1 g) of pimecrolimus (3.5 g) medium chain triglycerides, (0.5 g) oleyl alcohol, (1.5 g) propylene glycol, (0.5 g) stearyl alcohol, (0.5 g) cetyl alcohol, mono- and di-glycerides, (0.5 g) sodium cetostearyl sulphate, (0.2 g) benzyl alcohol, (0.05 g) citric acid anhydrous and purified water to 10 g.

All synthetic mixtures were extracted with 100 ml methanol, filtered, and the first 10.0 ml of the filtrate was rejected. Aliquots of the filtrate were diluted with the same solvent to obtain serial dilutions in concentrations ranging from (3-27 ng/ml), (0.004-60 µg/ml), (5-300 µg/ml), (0.08-5 ng/ml) of Naltrexone, ketotifen, oxcarbazepine and pimecrolimus respectively.

Assay solutions of drugs in their pharmaceutical preparation

The mixed contents of finally powdered 20 tablets were accurately weighed and finely powdered. Aportion of the powder, equivalent to one tablet of naltrexone, ketotifen or oxcarbazepine was weighed and transferred with the aid of several portions of naltrexone, ketotifen and oxcarbazepine respectively.

A measured volume of syrup equivalent to (1 mg) of ketotifen was transferred to 100 ml volumetric flask and the volume was completed with methanol. Aliquot of these solution were diluted with the same solvent to obtain (40 ng/ml).

A measured volume of eye drops equivalent to (1 mg) ketotifen was transferred to 100 ml volumetric flask and the volume was completed with methanol. Aliquots of these solutions were diluted with the same solvent to obtain (20 ng/ml).

A measured 10 gm of the cream equivalent to 100 mg pimecrolimus was transferred with the aid of several portions of chloroform to 100 ml volumetric flask and the volume was completed with the same solvent. Aliquots of this solution were diluted with the same solvent to obtain (200 ng/ml).

Assay solutions of drugs in spiked human plasma samples

Serial standard solutions of the drugs

Serial standard solutions were prepared in water (for I) and in methanol (for II, III and IV) to contain concentrations ranging from 2

to 50 ng/ml, 0.1 to 20 ng/ml, 0.5 to 60 µg/ml and 0.2 to 20 ng/ml for I, II, III and IV respectively.

Preparation of spiked human plasma samples

Two hundred µL of each serial standard solution containing I, II, III and IV were diluted with 1800 µL human plasma and vortex mixed to obtain concentrations ranging from 1 to 30 ng/ml, 0.04 to 18 ng/ml, 1 to 55 µg/ml and 0.1 to 10 ng/ml for I, II, III and IV respectively.

Preparation of assay solutions of drugs in plasma

Two hundred µL of each spiked human plasma samples of I, II, III and IV were mixed with 1800 µL methanol and centrifuged for 15 minutes to separate the precipitated protein. The clear supernatant was filtered through Millipore filter '0.45 µm' to obtain solutions in concentrations ranging from 0.5 to 15 ng/ml, 0.5 to 15 ng/ml, 5 to 40 µg/ml and 0.08 to 5 ng/ml for I, II, III and IV respectively.

Determination of I in volunteer's blood.

Blood sample was withdrawn in a test tube to which heparin was previously added and dried. The sample was centrifuged to separate plasma and then treated as previously mentioned under preparation of assay solutions of I in plasma samples (cf. preparation of assay solutions of drugs in plasma samples)

N^1-methylnicotinamide chloride reagent (NMNCl).

10 mM solution of NMNCl reagent was prepared by dissolving (1.7262 g) in one liter of 10^{-4} M HCl. Aliquots of this solution were diluted with distilled water to obtain (4.5×10^{-1}, 4×10^{-1} and 4×10^{-2} mM solutions.

General fluorometric procedure

One ml of each drug standard solution, assay solution of synthetic mixtures, assay solution of pharmaceutical preparations, assay solution of plasma samples or the assay solution of volunteer's plasma was transferred to 10.0 ml screw capped test tube.

Solutions of sodium hydroxide and NMNCl were added. The mixture was cooled in ice for indicated time. The pH of the solution was adjusted using formic acid and heated for the indicated time (TABLE 1) finally the solution was cooled in ice for 5.0 minutes.

The mixture transferred to 10.0 ml volumetric flask and the resulting solution was adjusted to volume with distilled water. Optimal concentration and volume of sodium hydroxide and NMNCl solutions, reaction pH, cooling time and heating time as well as λ_{ex} and λ_{em} were determined as shown in (TABLE 1). The fluorimetric measurements were performed against reagent blank experiments. Concentrations of each drug were calculated from the corresponding regression equations.

RESULTS AND DISCUSSION

When I, II, III, and IV were allowed to react with NMNCl under the optimal conditions specified for each, strong fluorescent products were obtained. The optimal wavelengths of excitation and emission of the reaction product were determined using synchronous wavelength search (TABLE 1).

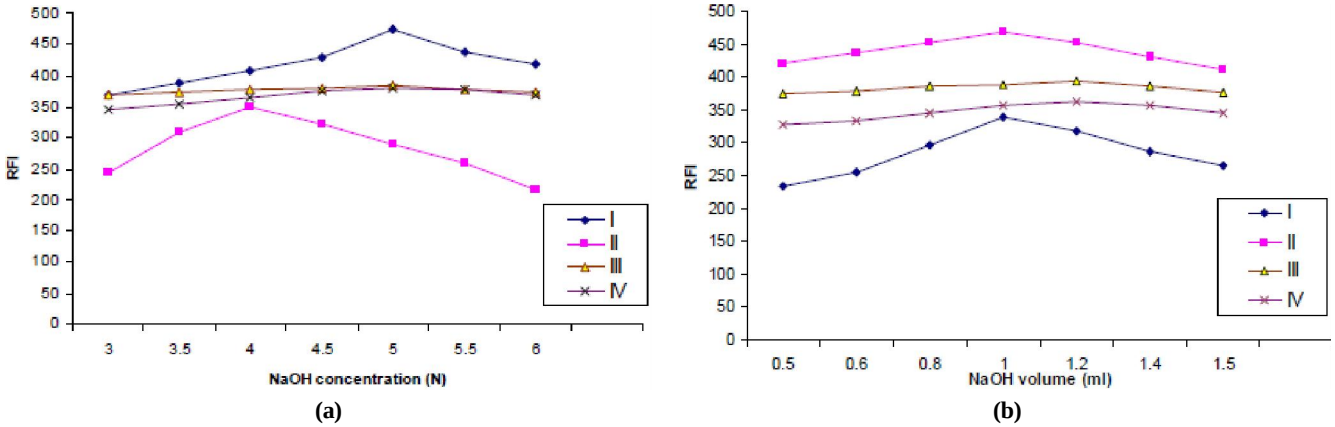
Different parameters analyzed are found to be affecting the reaction between the chosen drugs and NMNCl, including; (a) sodium hydroxide concentration and volume (b) volume and concentration of NMNCl, (c) pH values of the solution, were studied to optimize the reaction conditions to obtain a maximum fluorescence intensity (Figures 2, 3, and 4).

A linear relationships between the fluorescence intensities and the drug concentrations were obtained in the following ranges: 1–30 ng/ml, 0.04–15 ng/ml, 1–45 µg/ml, and 0.08–7 ng/ml for standard solutions of I, II, III, and IV respectively, and over concentration ranges of 2–25 ng/ml, 0.1–14 ng/ml, 3–40 µg/ml, and 0.1–8 ng/ml for spiked human plasma samples of I, II, III, and IV respectively. These results revealed good and dynamic linearity ranges of the proposed method with the analyzed drugs. The good linearity of the method was indicated by the corresponding regression parameters (TABLES 2 and 3) for standard solutions and spiked human plasma samples, respectively.

Validation parameters of the proposed method was studied according to the ICH topic Q2B (R1)

TABLE 1 : Optimum conditions for the fluorometric procedure

Drug	pH	NaOH Conc.(mM)	NaOH Volume (ml)	NMNCl conc. (mM)	NMNCl volume (ml)	Cooling time (min)	Heating time (min)	λ_{ex}(nm)	λ_{ex}(nm)
(I)	3	5	1.2	0.4	0.8	10	10	284	343
(II)	4.2	4.2	1	0.43	1.0	5	10	350	457
(III)	3	4.5	1.2	0.4	0.9	7	12	364	431
(IV)	3	5	1.2	0.04	1.0	10	9	331	433



(a) (b)

Figure 2 : Effect of NaOH concentration (a) and volume (b) on fluorescence intensity of the reaction products between Naltrexone (I) Ketotifen (II), Oxcarbazepine (III), Pimecrolimus (IV) and N[1]-methyl nicotinamide chloride respectively

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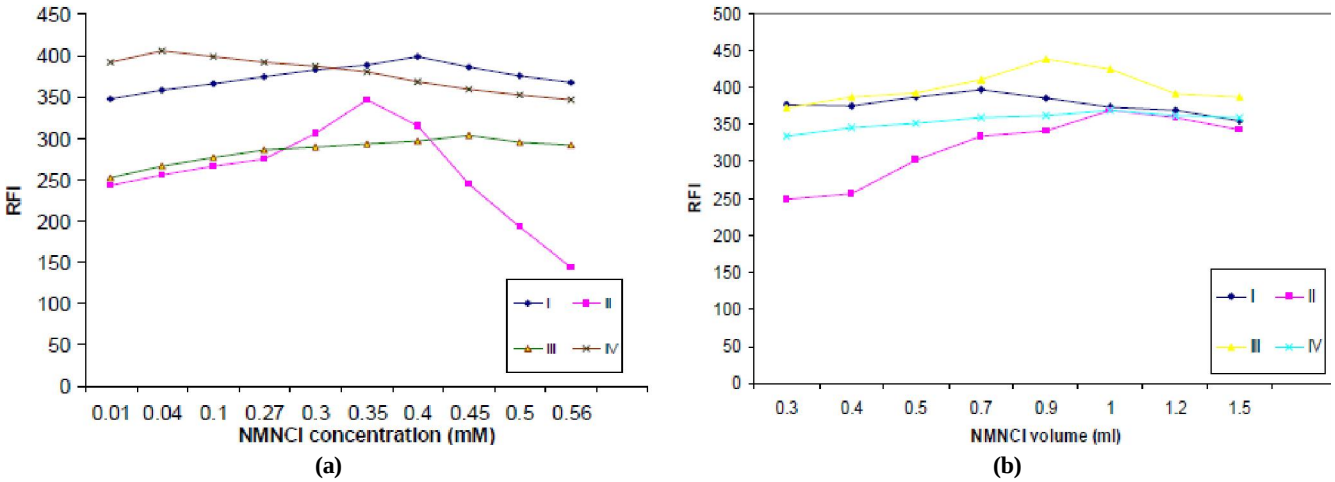

Figure 3 : Effect of N1-methyl nicotinamide chloride (NMNCl) concentration (a) and volume (b) on intensity of ûuorescent products resulted from the interaction between Naltrexone (I) Ketotifen (II), Oxcarbazepine (III), Pimecrolimus (IV) and N1-methyl nicotinamide chloride respectively

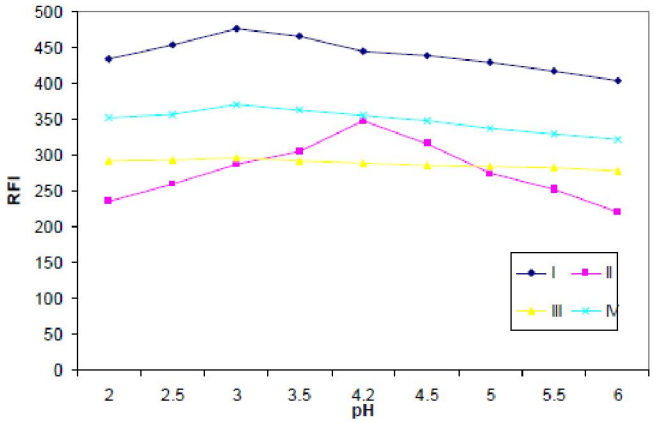

Figure 4 : Effect of pH on fluorescence intensity of the reaction and reaction products of Naltrexone (I) Ketotifen (II), Oxcarbazepine (III), Pimecrolimus (IV) with N[1]-methyl nicotinamide chloride (NMNCl) respectively

including;

Detection limit (DL)

Detection limits were practically determined and found to be 0.6 ng/ml, 0.01 ng/ml, 0.1 µg/ml, and 0.07 ng/ml, for standard solutions and 0.75 ng/ml, 0.03 ng/ml, 0.5 µg/ml, and 0.09 ng/ml, for plasma samples of I, II, III, and IV respectively.

Quantitation limit (QL)

Quantitation limits were practically determined and found to be 1.5 ng/ml, 0.05 ng/ml, 0.3 µg/ml, and 0.08 ng/ml, for standard solutions and 2 ng/ml, 0.1 ng/ml, 0.9 µg/ml, and 0.1 ng/ml, for plasma samples of I, II, III, and IV respectively.

Accuracy

The accuracy of the proposed method was studied by analyzing spiked human plasma samples. The results, expressed as % recovery ± S.D (TABLE 4) for spiked human plasma samples.

Precision

The precision of the method was judged by performing intraday and interday triplicate analysis of different concentrations covering the linearity range of each drug in spiked human plasma samples. The results are reported as S.D. and coefficient of variation (C.V.) (TABLE 5).

Specificity

To study the specificity of the proposed method, three synthetic mixtures of III and one synthetic mixture of I, IV and V were prepared to contain the possible interfering substances used during pharmaceutical formulations. These mixtures were analyzed using the proposed method, the results, were expressed as % recovery ± S.D., and were as follows: 100.35% ± 1.24 for I, 101% ± 0.04 for II, 100.2%± 0.01for III and 102.076% ± 4.56 for IV.

Assay of pharmaceutical preparations

All the pharmaceutical preparations available in the local market for each drug were analyzed using the proposed method. The results are expressed as % recovery ± S.D (TABLE 6).

TABLE 2 : Regression analysis parameters for the determination of I, II, III and IV in standard solutions

Drug	**Linearity range*	*Slope*		*Intercept*		R^2
		Mean	**S.E**	**Mean**	**S.E**	
I	1-30 ng/ml	7.645	0.034	262.21	0.875	0.999
II	0.04-15 ng/ml	31.553	0.07638	126.35	3.912	0.9990
III	1-45 µg/ml	4.674	0.043	337.36	2.650	0.9990
IV	0.08-5 ng/ml	9.642	0.340	307.4	2.987	0.999

*** Average of triplicate analyses**

TABLE 3 : Regression analysis parameters for the determination of I, II, III and IV in spiked human plasma samples

Drug	**Linearity range*	*Slope*		*Intercept*		R^2
		Mean	**S.E**	**Mean**	**S.E**	
I	2-25 ng/ml	5.347	0.256	268.48	1.540	0.999
II	0.1 -14 ng/ml	24.78	0.178	111.32	1.560	0.998
III	3-40 µg/ml	3.59	0.076	277.35	0.934	0.999
IV	0.1-5.6 ng/ml	47.54	1.567	321.95	2.543	0.999

*** Average of triplicate analyses**

TABLE 4 : Recovery data of I, II, III and IV in spiked human plasma samples

Drug	Claimed conc.	*Recovered conc.	% Recovery	Mean % recovery ±S.D.	C.V
I	3ng/ml	3.02	100.7%	100.33% ±1.612	1.607%
	6	5.97	99.47%		
	13	12.99	99.91%		
	15	14.97	99.82%		
	21	20.97	99.87%		
	27	26.95	99.82%		
II	1ng/ml	1.055	103%	103.4% ±4.949	4.786%
	3	3.058	100.23%		
	5	5.056	100.46%		
	7	7.039	100.44%		
	9	8.988	99.72%		
	10.5	10.536	99.52%		
III	2 µg/ml	2.534	94.45%	98.9% ±4.0079	4.053%
	7	6.946	97.685%		
	12	11.927	100.32%		
	17	16.690	100%		
	22	21.969	100%		
	32	31.806	99.768%		
	43	42.904	100.12%		
IV	0.2 ng/ml	0.2118	101.8%	100.7%±1.329	1.3198%
	0.5	0.5068	100.98%		
	0.9	0.9333	100.57%		
	1.4	1.4001	100.24%		
	1.8	1.7897	99.92%		

*** Average of triplicate analyses**

Determination of Naltrtexone (I) in Volunteer's Blood

The success in the application of the highly sensitive proposed procedure for the determination of (I) in spiked human plasma samples with good accuracy and precision, encouraged us to study its application for monitoring the drug level in the blood of a volunteer receiving (I) therapy. The level of (I) was monitored in the blood of volunteers, and its concentration was found to be 4.5 ng/ml which lies in the therapeutic levels of I (8.6 ± 4.5 ng/ml).

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TABLE 5 : Intraday and interday precision of I, II, III and IV determination in plasma samples

Drug	Claimed concentration	Found concentration	Intra day S.D	C.V	Found concentration	Inter-day S.D	C.V
I	3 ng/ml	3.0783 ng/ml	0.04949	1.608%	3.101	0.04056	1.3079%
	6	5.9533	0.04313	0.725%	5.999	0.06753	1.1257%
	13	13.013	0.05918	0.455%	12.994	0.05395	0.4152%
	15	14.911	0.05664	0.379%	14.936	0.02546	0.1705%
	21	21.051	0.05424	0.258%	21.001	0.04073	0.1939%
	27	26.981	0.02722	0.101%	26.969	0.04880	0.1809%
II	1 ng/ml	1.078 ng/ml	0.0516	4.791%	1.030	0.0629	6.097%
	3	3.064	0.0592	1.932%	3.008	0.0555	1.845%
	5	5.044	0.0363	0.721%	5.032	0.0176	0.349%
	7	7.044	0.0011	0.016%	7.015	0.0278	0.396%
	9	8.988	0.0383	0.426%	8.994	0.0335	0.373%
	10.5	10.567	0.0109	0.104%	10.52	0.0622	0.591%
III	2 µg/ml	1.932 µg/ml	0.1645	0.0851%	1.9540	0.0905	0.0463%
	7	6.923	0.0293	0.0042%	6.7915	0.1018	0.0149%
	12	12.12	0.0594	0.0049%	12.218	0.1874	0.0154%
	17	17.015	0.1025	0.0060%	17.024	0.0820	0.0048%
	22	22.218	0.1315	0.0059%	22.100	0.1982	0.0089%
	32	31.946	0.0905	0.0028%	31.927	0.0361	0.0011%
	43	43.076	0.2283	0.0053%	43.224	0.1327	0.0031%
IV	0.2 ng/ml	0.2433 ng/ml	0.0092	3.777%	0.2633	0.00919	3.49%
	0.5	0.5327	0.0071	1.340%	0.5513	0.01344	2.44%
	0.9	0.9213	0.0085	0.923%	0.9420	0.00707	0.75%
	1.4	1.4055	0.0056	0.398%	1.4124	0.01655	0.17%
	1.8	1.827	0.0049	0.268%	1.8560	0.00849	0.46%

TABLE 6 : Results of the recovery experiments of I, II, III and IV in different pharmaceutical preparations

Drug	Pharmaceutical preparations	*%Recovery ±SD
I	Deltrexone 50 mg tablet	100.57% ± 1.879
	Zedotefen 1 mg tablet	102.00% ± 1.23
	Zaditen 1 mg tablet	101.00% ± 0.26
	Zylofen 1mg tablet	100.85% ± 0.66
	Ketoti 1mg tablet	100.72%± 0.09
II	Zedotefen 1 mg/5ml syrup	100.00% ± 0.54
	Zaditen 1 mg/5mlsyrup	101.382% ±1.85
	Zylofen 1 mg/5ml syrup	101.00% ± 0.76
	ketoti 1 mg/5ml syrup	101.24%±1.82
	Zedotefen 0.025% eye drops	99.86% ± 1.26
	Zaditen 0.025% eye drops	101.16% ± 1.59
III	Trileptal 150 mg tablet	100.20%±1.49
	Trileptal 300 mg tablet	101.54 ± 0.54
	Trileptal 600 mg tablet	101.00% ± 1.25
IV	Elidel 1% cream	100.21%±0.036

CONCLUSION

The proposed method makes use of the highly sensitive and specific fluorometric technique to reach low limits of detection and quantitation for the studied drugs in active pharmaceutical preparation, synthetic mixtures, pharmaceutical preparations, spiked human plasma samples, and patient's or volunteer's blood. The method is simple; it gives results comparable to those obtained by other techniques that require elaborate instrumentation and time-consuming sample preparation procedure. The method showed good accuracy and precision suitable for quality assurance and could be recommended for bioequivalence and bioavailability studies as

well as for validation of cleaning methodology prior to line clearance of said dosage forms.

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