IJPSR (2016), Vol. 7, Issue 5 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 19 November, 2015; received in revised form, 14 January, 2016; accepted, 03 April, 2016; published 01 May, 2016

QUANTITATIVE DETERMINATION OF DRUGS IN BULK AND TABLET FORM BY USING TETRACYANOETHYLENE

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Key words:

Drugs, Tetracyanoethylene, charge transfer complexes, Quantification, and validation..

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ABSTRACT: The present work narrates UV-Visible spectrophotometric methods have been developed for the determination of selected drugs viz., Lamotragine (LMT), Venlafaxine hydrochloride(VFX), Valacyclovir hydrochloride(VCL) and Ractopamine hydrochloride(RAC) in bulk and their pharmaceutical dosage forms by using Tetracyanoethylene (TCNE) as reagent. These methods have been developed based on the formation of charge transfer complexes of drugs as n-electron donor with TCNE as π-acceptor. The selected drugs were formed yellow color complex with TCNE in Acetonitrile and exhibited a doublet at 400 & 420nm. Under the optimized experimental conditions, Beer, s law is obeyed over the concentration ranges of 2.5-12.5µg/ml for the all selected drugs. The effect of polarity of solvents, reagent concentrations, LOQ, LOD, and effect of reaction time have been studied and optimized. These methods have been validated in terms of ICH guidelines and applied to the quantification of selected drugs in bulk and dosage forms.

INTRODUCTION: Lamotragine: Lamotrigine, 6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine [1]. It is an anticonvulsant drug used in the treatment of epilepsy as partial seizures primary and secondary tonic-clonic seizures, and seizures associated with Lennox-Gastaut syndrome [2]. It is rapidly and completely absorbed with elimination half-life of about 24 hours of the administered dose, 70% can be recovered in urine 90% of which is in the form of glucuronide conjugate and 10% unchanged LMT [3].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.7(5).1985-90

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (5).1985-90

Through survey of literature reveals that a few analytical methods have been reported for estimation of Lamotrigine such as determination in biological fluids, using generally high-performance liquid chromatography (HPLC), high-performance thin-layer chromatography (HPTLC), liquid chromatography-tandem mass spectrometry(LC-MS=MS), gas chromatography (GC), and spectrofluorimetric [4] techniques.

Venlafaxine hydrochloride:
It is chemically designated as (R/S)-1-[2-(dimethyl amino)-1-(4 methoxy phenyl) ethyl] cyclohexanol hydrochloride [5], Venlafaxine (VEN) is a commonly prescribed antidepressant which is also prescribed and registered for treatment of anxiety and panic disorders [6]. Through survey of literature reveals that a few analytical methods have been reported for estimation of Venlafaxine hydrochloride like

liquid chromatography (LC) with UV detection, LC with electro spray ionization mass spectrometry, LC with coulometric detection, LC with fluorimetric detection, LC with diode array detection, gas chromatography-massspectrometry (GC–MS) , LC-MS, LC–MS–MS, and for the estimation in serum by using LC, HPLC methods, HPLC-MS/ESI, voltammetry, Capillary Electrophoresis, UV and Visible Spectrophotometry [7].

Valacyclovir hydrochloride:

Valacyclovir is chemically (S)-2-[(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)methoxy] ethyl - 2-amino-3-methylbutanoate) is a hydrochloride salt of L-Valyl ester of acyclovir. It is an oral antiviral drug used to treat infections with herpes zoster (shingles), herpes simplex genitalis (genital herpes), and herpes labialis (cold sores) [8, 9, 10].

Valacyclovir1 is an anti-viral, prodrug an esterified version of aciclovir that has greater oral bioavailability (about 55%) than acyclovir (10–20%). Valacyclovir is converted by esterases to the active drug acyclovir, as well as the amino acid valine, via hepatic first-pass metabolism. [11].

Through survey of literature reveals that a few analytical methods have been reported for estimation of Valacyclovir hydrochloride like Spectrophotometric methods, HPLC methods, and LC-MS methods for biological fluids [12]

Ractopamine hydrochloride:

Ractopamine hydrochloride (RAC·HCl, MW 337.85, (1R*,3R*),(1R*,3S*)-4-hydroxy-R-[[[3-(4-hydroxyphenyl)-1-methylpropyl]amino]methyl]-benzenemethanol hydrochloride [13] is a phenolethanolamine β-adrenergic agonist that is used to improve weight gain, carcass leanness, and feed efficiency in livestock by diverting nutrients from fatdeposition to muscle tissue production [14]. At present there few methods have been developed for the determination of Ractopamine hydrochloride such as enzyme-linked immunosorbent assay, gas chromatography–mass chromatography, HPLC, (LC–MS, LC–MS/MS, UPLC–MS/MS) [15]. Thorough survey of literature on the selected drugs revealed that quantification using *TCNE* as analytical reagent has not been reported yet. This paper reports simple, direct, and sensitive spectrophotometric method for determination of selected drugs using TCNE as π-acceptor based on the formation of charge transfer complex.

Experimental:

Instrument: Shimadzu 2600 double beam UV-Visible spectrophotometer is used to record the spectra of individual components as well as the charge transfer complexes, using matched pair of Quartz cells of 10mm path length.

Materials: The Tetracyanoethylene was supplied by sigma Aldrich. The AR grade solvents *viz.*, acetonitrile, methanol and chloroform are supplied by SD Fine chem. Ltd. Mumbai, India. The drugs used in present study were procured from Hetero drugs pvt.Ltd. Hyderabad and the pharmaceutical dosage forms of drugs were purchased from the local market.

Preparation of Standard Stock Solution for "VFX, VCL and RAC":

An accurate weight of drug (10mg) was weighed and dissolved in distilled water in a 10ml of validation flask and transferred into a 125ml separating funnel, where 5 ml of 0.1N NaOH solution were added .The mixture was mixed and extracted three times with 10ml of $CHCl_3$ each, then the chloroform layer was separated and evaporated to dryness where they obtained residue was dissolved quantitatively in 10ml of acetonitrile (final conc. 1mg/ml).

Preparation of Standard Stock Solution for LMT:

An accurate weight of drug (10mg) was dissolved in 10ml of acetonitrile to give a concentration of 1000µg/ml. The prepared standard stock solutions were further diluted according to the requirement for their analysis.

Determination of drugs in dosage form:

Lamotrigine: Ten tablets were weighed, finely powdered and an accurately weighed sample of powdered tablets equivalent to 50mg of Lamotrigine was transferred into 50ml validation flask and dissolved in about 50ml of methanol. The contents of flask were sonicated for 10 minutes.

The mixture was filtered and evaporated to dryness. Residue was dissolved in acetonitrile and then diluted to obtain a concentration in the range of linearity previously determined with pure drug.

Venlafaxine hydrochloride:

Ten tablets were weighed, finely powdered and an accurately weighed sample of powdered tablets equivalent to 50mg of Venlafaxine hydrochloride was dissolved in 50ml of distilled water in a 50ml calibration flask and transferred into a 125ml separating funnel, where 10ml of 0.1N NaOH solution were added. The mixture was mixed and extracted three times with 15ml of $CHCl_3$ each, and then the chloroform layer was separated and evaporated to dryness where they obtained residue was dissolved and then diluted to obtain a concentration in the range of linearity previously determined with pure drug.

Valacyclovir hydrochloride:

Fifteen tablets were weighed, finely powdered and an accurately weighed sample of powdered tablets equivalent to 50mg of Valacyclovir hydrochloride was dissolved in 50ml of distilled water in a 50ml calibration flask and transferred into a 125ml separating funnel, where 10ml of 0.1N NaOH solution were added. The mixture was mixed and extracted three times with 15ml of $CHCl_3$ each, and then the chloroform layer was separated and evaporated to dryness where they obtained residue was dissolved and then diluted to obtain a concentration in the range of linearity previously determined with pure drug.

Ractopamine hydrochloride:

Ten tablets were weighed, finely powdered and an accurately weighed sample of powdered tablets equivalent to 50mg of Ractopamine hydrochloride was dissolved in 50ml of distilled water in a 50ml calibration flask and transferred into a 125ml separating funnel, where 10ml of 0.1N NaOH solution were added. The mixture was mixed and extracted three times with 15ml of $CHCl_3$ each, and then the chloroform layer was separated and evaporated to dryness where they obtained residue was dissolved and then diluted to obtain a concentration in the range of linearity previously determined with pure drug.

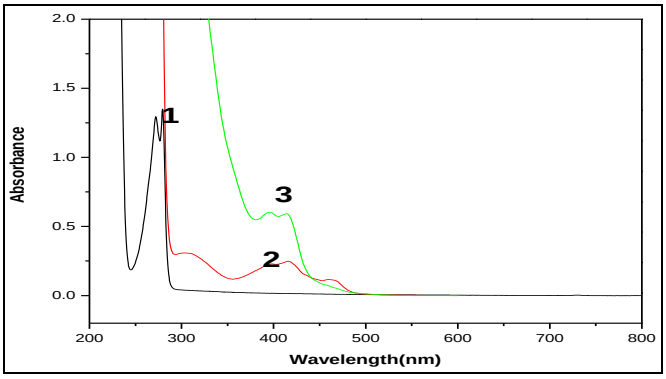

FIG. 1: (1) DRUG IN ACETONITRILE, (2) TCNE IN ACETONITRILE AND (3) CHARGE TRANSFER COMPLEX OF VENLAFAXINE HYDROCHLORIDE WITH TCNE.

RESULTS AND DISCUSSION: The TCNE solution of 3.125×10^{-3}M in acetonitrile was freshly prepared. Aliquots of drugs (0.5-2.5;50µg/ml) were transferred into a series of 10ml calibrated flasks, to each flask, 1.5 ml of TCNE solution in acetonitrile was added and remaining volume was made up by acetonitrile. The absorbance of yellow colored solution was recorded after 20min of mixing against reagent blank at 400nm and 420 are plotted against the corresponding concentrations (µg/ml) of the drug to construct the calibration curve.

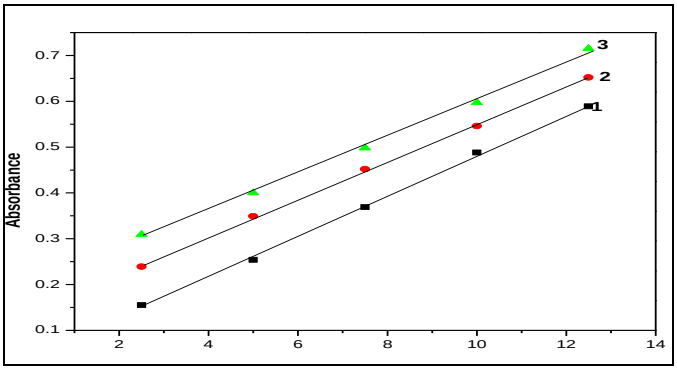

FIG. 2: CALIBRATION CURVES OF TCNE WITH (1) LAMOTRIGINE (2) VENLAFAXINE AND (3) RACTOPAMINE.

Effect of Concentration of Acceptor:

To establish the optimum concentration of reagent, Lamotrigine 12.5 µg/ml, Venlafaxine hydrochloride 10µg/ml, Valacyclovir hydrochloride 10µg/ml and Ractopamine hydrochloride 7.5µg/ml were react with different volumes of TCNE (3.125×10^{-3}). The results showed that the highest absorbance was obtained with 1.5ml. Hence 1.5ml of reagent was used for the determination of drugs.

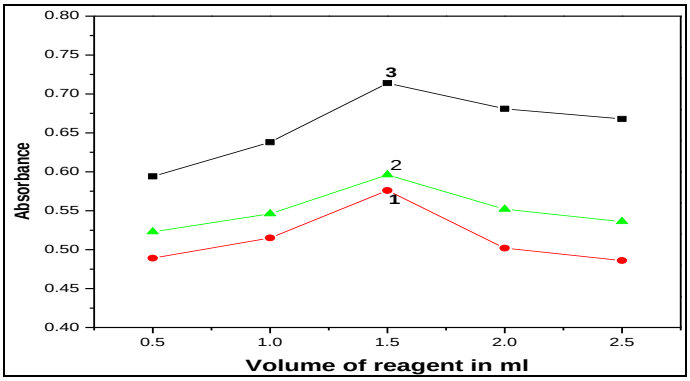

FIG. 3: EFFECT OF VOLUME OF REAGENT ON THE OPTICAL DENSITY OF THE ION - PAIR COMPLEX OF TCNE AND (1) LAMOTRIGINE, (2) VENLAFAXINE AND (3) VALACYCLOVIR.

Effect of reaction time:

The interaction of TCNE with drugs resulted in the formation of ion- pair complexes which stabilized with in 20 min of mixing. The developed color remained stable at room temperature for about an hour. After a day all solutions are decolorized.

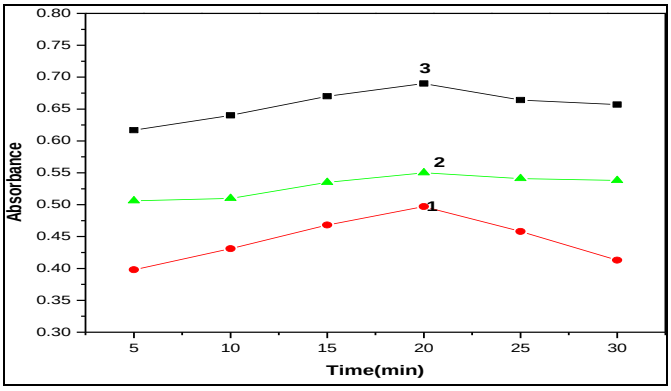

FIG. 4: EFFECT OF REACTION TIME ON FORMATION OF CHARGE TRANSFER COMPLEXES OF TCNE AND (1) RACTOPAMINE, (2) VALACYCLOVIR AND (3) LAMOTRIGINE.

Validation of the Proposed Method:

The methods developed have been validated in terms of guidelines of international conference of harmonization *viz.*, selectivity, sensitivity, precision, accuracy, linearity, LOD, LOQ. Sandell's sensitivity and robustness. The precision is tested by repeating each experiment at least 6 times while the accuracy has been tested by taking known weight of sample and performing recovery experiments. The robustness of the method was examined by performing the experiments on three different spectrophotometers with excellent tally of absorbance values. These methods developed have been applied for the analysis of pharmaceuticals. The recovery experiments performed show high accuracy and precision and the results are compared with the available validated reported methods on these drugs .The values % RSD and t- and F tests are in the permissible range of experimental errors. And show that the methods can be used in both pharmaceutical and drug industries.

Stoichiometry:

The Stoichiometry of each of the complex has been determined from Job's continuous variation method and found to be 1:1 in each case. A typical Job's plot of selected drugs with TCNE is presented in (**Fig.5**).

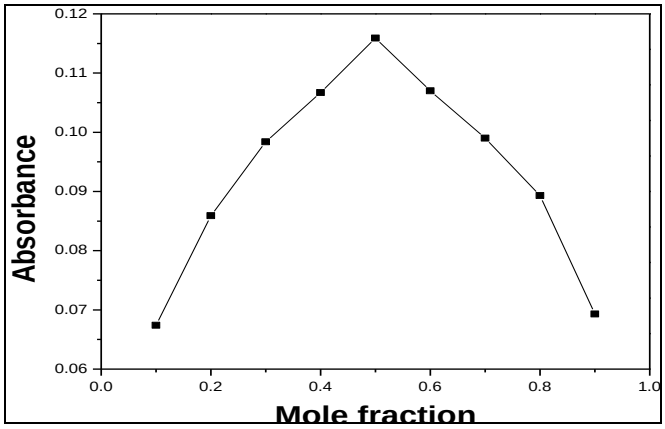

FIG. 5: JOB`S CONTINUOUS VARIATION PLOT

TABLE 1: SPECTRAL, ANALYTICAL AND STATISTICAL PARAMETERS OF CHARGE TRANSFER COMPLEXES OF DRUGS WITH TCNE

Drugs name parameters	Lamotrigine	Venlafaxine	Valacyclovir	Ractopamine
λ max, nm	400 & 420	400 & 420	400 & 420	400 & 420
Beer's law limit (µg/ml)	2.5	2.5	2.5	2.5
Molar absorptivity L mol^{-1} cm^{1}	48800	54600	55300	59700
Slope(b)	0.0451	0.0418	0.0164	0.0411
Intercept, a	0.03	0.1379	0.3871	0.1940
Correlation coefficient, r	0.9994	0.9989	0.9995	0.999
Sandell's sensitivity (µg cm^{-2})	0.0221	0.0239	0.0609	0.0243
Standard deviation of intercepts (n=6)	0.0032	0.0013	0.0036	0.0019
LOD (µg/ml)	0.2341	0.1026	0.7243	0.1525

LOQ (µg/ml)	0.7095	0.3110	2.1951	0.4622
Regression equation y=a+bx; x=concentration of drug(µg/ml)	0.03 + 0.0451x	0.1379 + 0.0418x	0.3871 + 0.0164x	0.1940 + 0.0411x

TABLE 2: APPLICATION OF PROPOSED METHOD FOR THE ANALYSIS OF THE STUDIED DRUG IN THEIR PURE FORM.

	Lamotrigine	Venlafaxine	Valacyclovir	Ractopamine
Amount taken(µg/ml)	2.5	5	5	2.5
	5	7.5	7.5	7.5
	7.5	10	10	10
	10	12.5	12.5	12.5
Amount found((µg/ml)	2.50	5.04	4.99	2.53
	5.02	7.49	7.52	7.51
	7.51	10.02	10.06	10.04
	10.01	12.52	12.47	12.56
% of Recovery	100.00	100.80	99.80	101.21
	100.40	99.86	100.27	100.13
	100.13	100.20	100.60	100.40
	100.10	100.16	99.76	100.48
% of RSD	0.17	0.38	0.40	0.45
	0.16	0.39	0.44	0.38
	0.19	0.37	0.47	0.36
	0.16	0.33	0.50	0.40
Proposed Mean ± SD	99.96±0.17	100.25 ±0.39	100.05±0.40	100.55±0.46
Reference Mean ± SD	99.40± 0.45	99.78±0.28	100.34 ± 0.39	105± 0.94
t-test	1.42(3.182)			1.12 (2.447)
F-test	0.14 (5.284)	1.94 (2.242)	1.05 (3.054)	0.2394 (3.054)

TABLE 3: APPLICATION OF PROPOSED METHOD FOR THE ANALYSIS OF STUDIED DRUGS IN THEIR PHARMACEUTICAL FORM.

	Lamotrigine	Venlafaxine	Valacyclovir	Ractopamine
Amount taken(µg/ml)	5	2.5	2.5	2.5
	7.5	7.5	5	5
	10	10	10	7.5
	12.5	12.5	12.5	10
Amount found((µg/ml)	5.01	2.51	2.51	2.50
	7.50	7.49	5.03	5.02
	9.98	10.03	9.99	7.51
	12.51	12.52	12.54	10.02
% of Recovery	100.20	100.40	100.40	100.40
	100.00	99.86	100.60	100.40
	99.80	100.30	99.90	100.13
	100.08	100.16	100.32	100.2
% of RSD	0.15	0.22	0.28	0.12
	0.16	0.32	0.34	0.17
	0.18	0.37	0.38	0.19
	0.19	0.27	0.29	0.14
Proposed Mean ± SD	100.2 ± 0.16	100.18± 0.23	100.30± 0.29	100.28± 0.13
Reference Mean ± SD	98.51± 0.24	99.98± 0.57	100.11± 0.14	99.00± 0.16
t-test	0.68 (3.182)	1.35 (2.145)		0.24(2.447)
F-test	0.44(5.284)	0.16 (3.054)	4.29(3.054)	0.66(5.284)

CONCLUSION: TCNE forms charge transfer complexes with selected drugs and exhibits doublet at 400nm and 420nm. The interaction enabled the quantitative determination of these drugs. This method is validated in terms of precision, accuracy, linearity and robustness. This method relies on the use of simple and inexpensive chemicals and techniques but provide sensitivity comparable to that achieved by sophisticated and expensive techniques like HPLC and LC-MS. Thus, they can be used as alternatives for rapid and routine determination of bulk sample and tablets.

ACKNOWLEDGEMENT: The authors are thankful to the Head, Department of chemistry for providing facilities. One of the authors B. Srinivas is thankful to CSIR New Delhi, for award of SRF.

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How to cite this article:
Srinivas B, Yadagiriswamy P and Venkateshwarlu G: Quantitative Determination of Drugs in Bulk and Tablet Form by Using Tetracyanoethylene. Int J Pharm Sci Res 2016; 7(5): 1985-90.doi: 10.13040/IJPSR.0975-8232.7(5).1985-90.